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(and related receiving waters)

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INTRODUCTION

The OECD Environment Committee has approved an OECD Programme Project on biological testing of effluents. Phase I of this effort (a voluntary phase funded by contributing member countries) culminated in a September 1984 workshop held in Duluth, Minnesota, the United States of America. About 40 representatives from ten countries, the Commission of the European Communities and the OECD Secretariat attended the workshop.

The overall goal of the workshop was to produce useful input for the 1985 OECD Project on Guidance for the Use of Biological Tests in Decision Making for Water Pollution Assessment and Control. The workshop took the form of 17 presentations followed by the preparation of three subgroup reports. The topics of the subgroup reports were as follows:

Subgroup I	- Application of Biological Testing Approaches and the Structure of OECD Guidance
Subgroup II	- Matters to Take Into Account When Using Biological Testing Data to Make Administrative Decisions
Subgroup III	- Scientific Considerations in Composing a Biological Testing System.

This document includes the three subgroup reports as developed at the Duluth workshop and a compendium of the presentations. The subgroup reports and the papers have not been edited. They represent the documents as presented at the workshop. The workshop organizing committee decided that it was more important to print and release the document quickly than to engage in an extensive editing exercise. Furthermore it must be recognized that the views expressed in all the material represents the individual views of the authors and does not necessarily represent the views of their country or organization.

The OECD Programme Project which commences in 1985 represents Phase II of the project. The focus of the project will not be on the development of biological testing per se but on the development of guidance on the use of biological testing for cost effective analysis and control of toxic effluents. The project is intended to help OECD member countries identify new approaches to the analysis and control of toxic effluents that are not only less costly than current approaches but also more environmentally effective. Specifically the project has two objectives: (1) to enable OECD member countries to gain an improved understanding of the usefulness and cost-effectiveness of biological testing and various biological testing methods and (2) to publish OECD guidance on the use of biological testing for cost effective analysis and control of toxic effluents, in particular how best to construct a biological testing system for a given decision situation.

The workshop presentations and subgroup reports represent a solid basis upon which to meet the objectives of the OECD Programme Project which begins in 1985.

SUBGROUP I - APPLICATION OF BIOLOGICAL TESTING APPROACHES AND THE STRUCTURE OF OECD GUIDANCE

INTRODUCTION

1. The "guidelines" described as the end-point of the OECD project need to be clearly distinguished from the OECD Chemical Guidelines. The key distinction is that the Chemical Guidelines consist of recommended methods. The guidelines envisioned for the biological testing project will not consist of a compilation of methods but rather will convey principles for decisions about use of the available testing methods in different kinds of decision scenarios (control development, planning and priority setting, effects monitoring, etc.) (Many, if not most, of the test methods will be similar to the single chemical tests.) In order to avoid confusion with the Chemical Guidelines Program, the working group recommends that the resulting product of the biological testing project be termed "Guidance for the Use of Biological Tests in Decision-Making for Water Pollution Assessment and Control".

2. Working groups 2 and 3 will provide basic comments on the findings of the workshop with respect to the scientific and regulatory issues. The following sections outline thoughts of Working Group 1 on key points but the work of all three groups needs to be synthesized for a complete statement in such areas as "Benefit", "Limitations", and "Restraints".

Subgroup 1 Conclusions

Subgroup 1 agreed:

- that biological testing approaches have application in OECD Member Countries and that it would be useful to have an OECD Guidance Document on the use of biological testing.

Subgroup 1 agreed:

- that the purpose and benefits of the Guidance Document would be
  - to provide information as to what knowledge exists and what knowledge does not exist to help in making decisions;
  - to hasten the implementation of biological testing in Member Countries; and
  - to bring about the common application of biological testing that would help bring increased harmony among OECD Member Countries (in regard to the level of environmental protection and industrial pollutant control among Member Countries) and have a positive effect on the impact of pollution control on the competitive status of certain industries in international trade.

Subgroup 1 also agreed, however:

- that various Member Countries have differing characteristics (both among countries and internally) that are important to the application of biological testing and guidance. Therefore, biological testing guidances in recognizing these characteristics should consider the following conditions:
  - pollution problems
  - pollution control legislation
  - the nature of regulatory or administrative agencies
  - technology
  - industries
  - national economies
  - types of receiving waters
  - personnel skill and knowledge levels

Therefore, Subgroup 1 agreed:

- to suggest the following structure of the Guidance Document:
  - Chapter 1 Introduction of Background Facts
  - Chapter 2 Policy Considerations in the Application of Biological Testing
  - Chapter 3 Principles on How to Compose a Test System
  - Chapter 4 Interpretation of Test Results for Decision-Making
  - Chapter 5 General Conclusions and Recommendations
  - An Appendix could be prepared to contain the supporting detailed information, since it is important that the Guidance Document be concise and that prominance be given to key points so that it will be useful to decision-makers and administrators.

Subgroup 1 agreed that the following factors deserve special consideration in preparing the OECD guidance:

Purpose and Benefits

There have been reservations among regulators to rely on biological testing to the extent that this subgroup believes that guidance needs to be given. Therefore, the OECD guidance needs clearly to outline the benefits of biological testing in a variety of decison-making scenarios.

Key purposes of biological testing appear to be:

- to regulate effluents
- to define the need for control
- to assess effectiveness of controls
- to detect potential problems (possibly early warning of human health effects).

Key benefits of biological testing seem to be (not in order of priority):

- a variety of biological testing methods are available for complex effluents. These methods are in harmony with the OECD Chemical Guidelines.

- Biological tests allow assessment of the biological impact of effluents on receiving environments. This assessment includes the consequences of the biological availability of toxic substances; the assessment of short and long-term biological consequences and possibly the assessment of their potential for becoming health hazards through the food chain.

- For a complex effluent, biological tests may provide a less expensive and quicker assessment of toxicity and ways to control toxins than chemical testing.

- Biological tests provide perceptible results which may be more convincing than chemical tests, in demonstrating that controls are needed.

  The number of treatments and control techniques available is less than the very large number of elements that could be toxic (i.e. the polluting properties of the effluent need not be precisely defined in order to treat it).

- Continuous biological monitoring of the effluent provides rapid feedback often in time to implement control processes.

Limitations

To be useful to decision-makers, the guidance needs to articulate the limitations in the use and interpretation of biological tests. Understanding when to use what tests is the key to effectiveness in decision-making as well as to the credibility of the testing program. Some of the key limitations are listed below (to be supplemented by the contributions of Subgroups 2 and 3):

Biological testing of effluents cannot be used as the sole means of assessing environmental impact and of developing controls. For example:

- Biological tests may not readily detect long-term lethal or sublethal effects (somewhat test-dependent).

- The linkage between performance in an effluent toxicity test and downstream effects needs further work.

- Biological tests of complex effluents have limited ability at this time to assess human health effects, thus requiring association with other tests.

Relationship to Other Tests (e.g. chemical testing, field surveys)

If only relative assessments are intended (i.e. comparing relative toxicity of individual plants for priority-setting purposes), only limited complementary tests or studies are needed. However, if one is attempting to perform an environmental assessment, field surveys or testing of individual chemicals may be needed. It is essential that the guidance articulate principles for where, when and how additional information and complementary analytical approaches need to be considered.

Restraints and Requirements

The Guidance needs to describe those restraints and considerations which make Biological Testing successful, for instance:

- appropriately trained personnel
- availability of laboratory resources
- stable and even work load in laboratories which perform the tests
- the proper use of criteria for the selection of cost effective types of tests for different levels of decisions
- mechanisms for standardization and intercalibration
- sufficient time for advance planning
- nature of the testing environment as it bears on selection of test organisms and protocol.

Subgroup 1 agreed:

. that the next step is for input from Subgroups 2 and 3 to be synthesized with these Chapter suggestions to identify the specific key elements of these chapters.

SUBGROUP II - MATTERS TO TAKE INTO ACCOUNT WHEN USING BIOLOGICAL TESTING DATA TO MAKE ADMINISTRATIVE DECISIONS

INTRODUCTION

This subgroup was asked to examine matters that should be taken into account when using biological testing data to make administrative decisions. The subgroup expanded its mandate to include administrative issues in general as they relate to the application of biological testing.

To facilitate the discussions within the subgroup, our observations were divided into three categories depending on the type of administrative decision required. The three categories are described below.

a) Problem identification/priority setting

This item covers a number of aspects including:

- The selection of the most significant dischargers of toxic chemicals into a given receiving waters,
- the selection of the most important industrial sector for regulatory, intervention,
- screening manufacturing processing options to select the least harmful option, and
- the selection of the most significant discharger of toxic chemicals within a given industrial sector.

b) Regulatory initiatives

This item covers the use of biological tests to set a legally binding requirement on a point source discharge.

c) Monitoring

This item refers to the use of biological tests of effluents as well as the receiving environment. This involves the evaluation of compliance with a regulatory initiative(s), as well as systematic monitoring of a plant that may be unregulated.

GENERAL CONCLUSIONS

- Biological test results on effluents are considered as valid, cost effective inputs to decisions related to the protection of the aquatic environment.

- Toxicity results must be considered in the context of other information (i.e., results from ambient tests and surveys, chemical and physical properties, the size and nature of the receiving environment and economic and social factors) that form logical components of a decision-making framework.

- Most countries have instituted control programs based on technological achievability. These minimum treatment standards should be maintained regardless of whether biological testing (or chemical) methods show no impact.

- Given the subgroup's agreement that biological tests are a valid input to decision-making a logical question to ask is "Why are the biological results not being used more extensively in the decision-making process in OECD Member Countries?". The subgroup concluded that there are three reasons. First, there has historically been a lack of generally accepted test methods. This is no longer a concern. Second, the regulatory focus in most countries has been on technology-based standards and not on water quality. Third, there is often a lack of understanding and cooperation among administrators who must make decisions and the scientists who provide biological test data. The onus for improvement rests on both parties (the scientists and the managers).

- Education of decison-makers, the public, and private industry on the ecological relevance of toxicity data is required. On the other hand, scientists must speak with a more common voice when providing input to decision-makers. For example, debates over minor sensitivity differences between species do not instill the decision-maker with great confidence. Efforts should also be made to express toxicity data in quantitative terms. Decision-makers are inherently more comfortable with such an approach.

PROBLEM IDENTIFICATION/PRIORITY SETTING

- It is believed biological tests are more cost effective in many cases than chemical analysis. This needs to be substantiated by the OECD program.

- Provided sensitive tests are used, the test species used are not especially important but biologists need to agree on which tests to employ.

- Effluent testing can generally be used alone although in some cases ambient toxicity tests should be used.

- Testing and evaluation should in the first phases involve a low level of effort and the use of existing information is important.

- There was agreement that scientific accuracy is not a major issue as compared to regulatory uses.

- Health-related biological tests (such as Ames tests) on effluent samples provide a measure of "biological reactivity" but no direct connection to health risks can be made. There was a feeling that testing should continue even if our ability to interpret this data is still developing.

REGULATIONS

- A major benefit of using effluent toxicity as a regulatory control parameter is administrative simplicity compared to testing and controlling numerous chemicals.

- In some cases the chemical approach is more cost effective. The toxicity testing approach becomes more important as the complexity of the effluent increases.

- Administrators must be willing to accept some uncertainty that controls based on biological tests will adequately protect the environment. Toxicity tests are generally more reliable than the chemical approach. There are several other sources of uncertainty more significant than the measurement of toxicity or chemical concentration.

- OECD should determine the range of the legal status of biological testing and examine means of circumventing identified restraints.

- Multiple source situations are difficult to address. A country's regulatory approach has a strong influence. Ambient toxicity testing may be useful to assist interpretation of effluent data.

- There was a lack of agreement regarding whether developing treatment strategies is more difficult for toxicity requirements. OECD needs to look at treatability assessment techniques and how they are most effectively used.

- A higher degree of scientific certainty is needed for regulatory purposes than for problem identification. Concern was expressed that currently used biological tests may not detect potential or subtle pollution effects. This concern is also true for chemical approaches.

- Governments need to develop internal testing capability and expertise. It is very important that funding should be consistent from year to year in order to retain personnel and increase expertise levels. This has been a problem in many countries represented in the subgroup.

- Simple and inexpensive tests should generally be used for regulation. Where controls are developed on a case-by-case basis, more expensive tests may be necessary in some cases.

- There was a lack of agreement about whether freshwater tests should be used to regulate discharges to salt water. Psychological factors may be relevant.

- Administrators should be ready to accept conclusions of no impact. The possibility should be left open to correct problems identified at a later date because there are many pollution effects that are not easily measured at this time.

MONITORING

- The biological testing approach is particularly valuable in detecting episodic events (e.g. spills and upsets). Long term trends are also best measured by biological testing of effluents and the ambient environment.

- Enforcement capabilities would be greatly enhanced using continuous toxicity measurement techniques. OECD should review the availability of such methods.

- Ambient toxicity tests could be used to monitor water bodies and define zones of influence.

- The value of employing biological testing as a tool to assist countries in transboundary and boundary situations should be reviewed in the OECD project.

- An important aspect of routine monitoring programs is to maintain and improve skills of testing personnel.

SUBGROUP III - SCIENTIFIC CONSIDERATIONS IN COMPOSING A BIOLOGICAL TESTING SYSTEM

I INTRODUCTION

Most of the problems and principles of whole effluent testing are common to those of single chemicals as well. However, factors that make testing wastewater samples different from testing single chemicals are: effluent variability (i.e. variation in chemical composition) unknown chemical composition and the number of constitutants. Largely because of this variability, assessments of effluent impact are site specific and specific data for a given effluent are not widely applicable, even to discharges of similar installations. This report confines itself to routine testing of effluents for regulatory purposes.

The following items have been excluded from this report:

Human Health Concerns

This subgroup report does not consider human health concerns but we note that even though there are a few methods, such as the Ames Test, there is a paucity of tests for health effects.

Carcinogenicity

Carcinogenetic effects on aquatic populations are not ecologically significant unless they occur in a high enough percent of the population to affect population numbers. Such effects may also reduce marketability or recreational value of a fishery. Lesser incidence but above normal background occurrence may be a signal to consider human health effects from consumption or exposure to the water.

Organoleptic Effects

Organoleptic effects are important for some effluent types but this effect is not due to toxicity and is not included in this report.

Relationship to Chemical Methods

Various methods such as quantification of single compounds and measurement of chemical/physical properties (e.g. partition coefficients) are considered to be chemical tests and we note that such tests are closely relatable to bioconcentration. The relationship between chemical tests and toxicity is much less established. For effluents that do not vary in chemical composition, and especially for effluents containing a small number of contaminants, chemical surrogates for toxicity may exist that can be more easily used for monitoring than can biological tests. The validity of such tests must be established however.

II METHODS REVIEW

Biological tests relative to effluent and receiving water assessments were classified into four major categories: single species, multi-species, field studies and suborganism. Review of the multi-species category indicates that there are insufficient data as to the validation of such ecotoxicological tests for routine effluent testing. Therefore, this category has been excluded at this time. Table 1 provides examples of the types of tests believed to be useful and as routine procedures to have adequate reproducibility. The level of difficulty (complexity and required expertise), cost (excluding capital costs) and interlaboratory calibration are estimated as low, moderate or high. These relative estimates are comparable to similar estimates that would be made on conventional chemical analyses ranging from dissolved oxygen measurements to gas chromatograph-mass spectrometer analysis.

III POINTS OF CONCENSUS

A range of skill is required to perform available tests depending on the type of test and the experience with it. In general the skill required is comparable to that necessary for the typical types of chemical tests performed on effluents. Equipment costs are also comparable.

Methods Available

A variety of methods is available in sufficient detail to allow ample testing of whole effluents to be conducted. Improvements can be expected to lessen cost and improve their value.

Reproducibility of Methods

Reproducibility of biological methods described in the table is similar to that for chemical methods used for effluent testing. When tests are performed by competent laboratories, the results can be reproduced with an acceptable degree of variation. This variation in test method reproducibility is orders of magnitude less than the variable encountered because of variation in flow rates and chemical characteristics of receiving waters and effluents.

Exposure Variability

Variations in effluent flow and characteristics and variation in stream flow and quality are large. Special attention must be exercised when sampling to obtain a representative sample. The problems however are no different than those associated with sampling for chemical analyses.

Resident Species

Having experience with testing a species is more important than whether they are locally found in the receiving water. The use of resident species is not necessary. Test species that resemble resident species in regard to feeding habits, physiology, and behavior are preferred.

Species Selection

A few well chosen test species are sufficient. The battery of test species should include ones that represent widely different taxanomic groups. The fish, algae and daphnid chronic battery of tests currently used by OECD for single chemicals is an example of a useful test battery. Knowledge of resident species sensitivity or effluent characteristics may be useful in species selection.

Hazard Assessment

Predicting impact of exposure from effluents encompasses the same elements as a hazard assessment for single chemicals. The principle information needed is the predicted or measured exposure and the acute and chronic toxicity. Estimation of exposure requires knowledge of persistence and the distribution within the receiving water (e.g. sediment, water column, tissue).

Interaction

Positive and negative interactions, for example additivity, are incorporated into whole effluent tests if the tests are conducted in a way to allow these interactions to be measured. However, bioavailability will change with time after dilution in the receiving water and such changes will not be measured in tests made on fresh effluents.

Tiered Approach

A tiered evaluation scheme should be used but users should be able to enter the scheme at any tier.

IV AREAS NEEDING MORE KNOWLEDGE

The following seven areas of research need are arranged in order of priority from highest to lowest.

* Field Validation

More studies are needed to document how valid the test methods are for predicting impact. The needs are the same as for single chemicals.

* Interlaboratory Calibration

Most of the available test methods need to have precision and accuracy determined through inter-laboratory calibration tests.

Distribution and Persistence

Better methods are needed to measure the compartmentalization and persistence of toxicity in the receiving water in order to accurately describe the environmental exposure occurring.

Bioconcentration Test

Tests exist to measure uptake into the animal body but better methods for measuring bioconcentratable substances from unknown mixtures are needed both for wastewater and tissue.

Cost-Effective Analysis

The cost-effectiveness of biological methods should be compared to that of chemical methods to identify in which situations the two types of tests can most effectively be used. The chemical methods compared must be proven to be accurately correlated with biological effects.

Marine Tests

Fewer tests methods are available for marine organisms than for freshwater organisms and more methods for marine species would be especially useful. These needs are equally true of single chemicals.

Toxicity Reduction Evaluation

Better methods are needed to enable one to locate the source of toxicity among the waste streams composing the final effluent. Particular attention must be given to comparisons involving treated final effluent and untreated component waste streams.

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* Items that are acceptable for OECD projects.

TABLE I - EXAMPLES OF TYPES OF BIOLOGICAL TESTS BELIEVED TO BE USEFUL AS ROUTINE PROCEDURES

TEST METHOD	ENDPOINT	LEVEL OF DIFFICULTY	COST	AMOUNT OF INTERLAB CALIBRATION COMPLETED
SINGLE SPECIES				
Fish acute	lethality	+	+	+++
Daphnid acute	lethality	+	+	+++
Fish acute (automated continuous)	operator rate	++	++	+
Fish acute (automated continuous)	swimming performance	+	++	+
Mussel acute (automated continuous)	valve closure response	+	+	+
Mysid shrimp acute	lethality	+	+	+++
Brown shrimp acute	lethality	++	++	++
Nitocra acute	lethality	+	+	+
Protozoa chronic	growth	++	+	++
Algal chronic	growth reproduction	+	+	+++
Daphnid chronic	reproduction	++	++	+++
Fish chronic (early life stage)	growth	++	++	+++
Fish chronic	physiological, biochemical	+++	++	++
Mussel chronic	growth, bioconcentration	++	++	+
Barnacle chronic (early life stage)	bysis formation, lethality	++	+	+
Terrestrial plants chronic	growth, distortion	+	+	+

TABLE 1 (CONT'D)

TEST METHOD	ENDPOINT	LEVEL OF DIFFICULTY	COST	AMOUNT OF INTERLAB CALIBRATION COMPLETED
Lemna chronic	growth	+	++	+
Mysid shrimp chronic	growth, reproduction	++	++	+++
Sea urchin chronic (early life stage)	reproduction	++	++	+
Nitocra chronic	reproduction	++	++	+
Bacterial lumnisence chronic	light inhibition	+	+	+++
FIELD EXPERIMENTS				
Caged organisms	lethality, growth, bioconcentration	++	++	NA
Indicator species	abundance	+	++	NA
Resident species	avoidance	+	+++	NA
Communities: artofocoa; sibstrates	species diversity species composition species richness	+++	++	NA
FUNCTIONAL TESTS	Primary production respiration biomass	+++	++	NA
SUBORGANISM				
Chemical tests	enzyme inhibition, stimulation	+++	++	++
Tissue	growth, lethality histophatological	+++	++	++

+ Low ++ Moderate +++ High

The Role of Biological Assessment in Effluent Control

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This workshop, as indicated by the title, is to examine the state of the art of using biological measurements to assess and control the biological impact of effluents discharged to surface waters. The conference organizers hope that the proceedings of the conference will identify the currently available techniques that are reasonably well proven as reliable ones and identify where we yet need development of additional methods or approaches to better use biological measurements for control purposes.

I would like to tell you about some recent progress in the U.S.A. in validating the predictability of effluent tests to predict the receiving water impact of complex effluents. I would also like to tell you about plans in the U.S. EPA to use effluent tests to limit the discharge of "toxicity" as a control requirement without specifying the substances causing the toxicity. As I describe these programs I am sure controversial issues will emerge that will provide discussion stimulus for this conference.

You should bear in mind that all of my comments stem from the situation and regulatory framework that now exists in the U.S.A. To the extent that these are different in other countries, may change the desirability or workability for those places where conditions are different. I would hasten to point out however that at least the regulatory framework is manmade and therefore can be changed. I firmly believe that it is our duty as scientists concerned with the quality of surface water and the ecosystem contained therein, to press for changes in the existing regulatory framework where we have reason to believe there is a better way to regulate. This conference occurs at a time of great satisfaction for me because some of the approaches I have long supported for control of complex effluents are now being implemented. I have had the privilege of working with some of the prominent names in water pollution efforts in this country of past years. I refer especially to Dr. Peter Doudoroff and Dr. Clarence Tarzwell who contributed so much to the thinking of this field in years past. I am sure much of my philosophy and approach was gleaned from them and I hope that I can be a part of the effort that implemented some of their early but excellent ideas about effluent control.

At this point I need to diverge, briefly I hope, to be sure you are aware of some of the salient features of the past 15 years of water pollution legislation and abatement progress in the U.S.A. The reason for this divergence is so that you will be better able to understand the reasons for some of our decisions and future plans.

Beginning in the early 1970s, the U.S.A. set forth on a program to require all dischargers to have a permit to discharge effluents into surface waters. Further, every discharger was to be required to install a specified degree of treatment whether the untreated discharge caused an adverse effect or not. It is important to note that the amount of treatment required had no relationship to the receiving waterbody - it was a uniform treatment requirement based on available treatment technology and its cost.

In addition, where that amount of treatment did not result in the elimination of adverse effect on the receiving water, additional treatment was to be required. I was not a party to the discussions that resulted in the decision but I am told that in part the reason to take this approach was that receiving water quality standards were too few, too controversial and too costly to enforce.

The uniform treatment approach set in motion what must have been the largest construction effort to build wastetreatment plants for the 70,000 plus discharges in the U.S.A. that has ever occurred anywhere. The legislation also said that while this construction was in progress, higher levels of treatment were to be defined and required in the decade of the 1980s. The original level of treatment has largely been completed and the main effort was to remove BOD, solids, control pathogenic bacteria and a few other commonly occurring pollutants. This treatment, although not primarily focused on removal of chemicals, especially organic chemicals, is as a side benefit removing a large amount of them. As a result a great many of our effluents no longer have a measurable acute toxicity. However, a substantial percentage do have measurable toxicity, especially chronic toxicity.

We are now to the time when the higher level of treatment is to be required. These higher levels termed "Best Available Treatment" (BAT) have been defined for some types of discharges but not for all types. Further, for some the cost is very high or the additional removal does not seem to warrant the cost. In early 1984, the U.S. EPA issued a "Water-Quality Based Policy" which places emphasis on giving priority attention to those discharges which are thought to be having the largest adverse impact on the receiving water rather than only blanket uniform treatment for all discharges. The policy further states that toxicity will be controlled in effluents as well as other characteristics. Toxicity limitation, is the part of the policy which is of great interest to me and is closely related to the subjects to be discussed by the participants of this conference. It is also completely consistent with some of the basic goals set forth in our Water Act which state that toxic pollutants in toxic amounts are to be prohibited and in another goal that among other things all waters are to be "fishable".

We currently have very few discharge permits which expressly limit toxicity emission in effluents although there are considerably more permits which require toxicity to be measured but do not limit the degree of toxicity. I have been involved in a research effort for the past several years to determine how well some effluent tests currently available will predict biological impact in the receiving water. I would now like to describe for you some of the insights and results we have gained in this effort.

Even though much pollution abatement effort is done for the protection of toxicity to aquatic life, the treatment systems required were rarely, in fact almost never, evaluated to see how much toxicity was removed. Little wonder then that many treatment plant effluents still are toxic. Of course the immense increase in synthetic organic chemical usage in recent years has contributed to this situation. At this point I should emphasize that it is the full intent of EPA to limit toxicity without expending the effort to identify the specific substances causing the toxicity. Many scientists and regulators are surprised or even disbelieving that limitation of toxicity is feasible without identification of the causative agents. Their attitude is of even greater surprise and disbelievement to me! After all did we ever or do we now need to know the specific components of suspended solids in order to remove them in treatment? Did we or do we now insist on knowing the specific components causing BOD before we can require its removal? Do we insist on knowing the specific chemicals that cause TOC (total organic carbon) before we place a limit on its discharge? The answers to the above questions are, "of course not" and limiting toxicity of an effluent is no different. "Generic" treatment requirements are commonly used.

For some reason many people seem to feel toxicity is different and the specific chemicals causing it must be identified. A reason sometimes given for this position is that we do not know how to design treatment systems to remove toxicity. There are several responses to this statement. Perhaps most forceful is that the way we learned how to remove BOD, SS and TOC was to try various treatment methods on various wastes and see how good the removal was. Toxicity removal can be determined exactly the same way. Now obviously some allowance of time will have to be given for this determination but we will never learn how to remove toxicity if we do not have the forcefulness to require its removal. At least in the U.S.A. and I suspect in some other countries as well, the regulatory agency does not specify how to remove toxicity or any other effluent characteristic. This discretion is left to the discharger. I mentioned earlier that even the relatively basic treatment now in place in the U.S. has removed much, very much toxicity. There is nothing to prohobit any discharger from consulting with other dischargers who do not have a problem to learn how removal might be accomplished. Furthermore, in my experience, dischargers are often very adept at locating probable in-plant sources, especially if a little persuation is added! Someone, whose name I cannot now recall once said, "It takes some courage as well as knowledge to abate water pollution."

While I see little short-term value in fractionating effluents as a chemical analyst would do, that is acid extractables, base extractables, etc., I see a lot of value in what I like to call waste engineering fractionation. By this I mean fractionating effluents using techniques that are directly relateable to waste treatment methods that might be employed. For example it takes little imagination or resources to run a sample of effluent through a bench scale carbon filter or to try a bench scale air stripping technique to see if toxicity is removed by such procedures. If it is, a treatment alternative becomes immediately obvious that will remove toxicity without identifying the causative agents. Frequently, some simple housekeeping procedures are found that remove the problem, especially where there is a good suspicion as to the source.

I am sure some of you who are chemists are saying "Why not just analyze the effluent and see what it contains?" There are several replies to this question. First, if anything beyond a very simple analytical measurement is needed, the cost of effluent toxicity tests now available is at least an order of magnitude less than the chemical analyses and if a complex analyses is needed, such as GC-MS, then the toxicity tests will be 2 or 3 orders of magnitude less. Even setting cost aside as a concern, a chemical analysis of a complex waste will usually show 10's, 100's or 1000's of substances present. One certainly cannot assume the substances at highest concentration are the problem. Neither will one have data on what the toxic concentrations are for more than a few in the list. And finally you will probably have no knowledge about which ones may be in a biologically active form or what ones will display interactions, either positive or negative.

I am sure the biologists are saying, "Why not use field biological surveys to measure impact rather than toxicity tests and avoid the necessary extrapolation?" The answer is much the same as for the previous question. The cost of a good biological survey is at least 2 and probably more than three orders of magnitude greater than toxicity tests in all but the simplest situations and smallest receiving water. Second, if there are multiple dischargers, the most common situation by far in the U.S., there is no chance to determine which discharge is causing how much effect except in rare instances and one certainly cannot measure how much toxicity reduction should be required to alleviate the problem. Thirdly, even the best field surveys fail to detect any but the the more gross effects due to toxicity so impacts must be large to detect their presence and finally, field studies require that the adverse impacts have ocurred in order to measure them and therefore are not useful for treatment decisions on new discharges.

I want to emphatically state that I nor others in the U.S. feel that we do not need chemical analyses, single chemical criteria or biological surveys. Chemicals that persist in the environment for long periods and are found in many places or in many non-point sources are best managed by single chemical criteria (standards). Chemical analyses, especially for groups of chemicals can often help to find the cause of toxicity and a treatment alternative. And biological surveys have a critical function to provide insight into long-term trends and the overall health of large reaches of receiving waters. Data from these types of measurements are hard to translate into permit language and we are saying, toxicity should be limited too.

Neither will toxicity tests on effluents provide all of the answers needed for control purposes. Changes in equilibrium resulting from fate processes, time and dilution are likely to be poorly estimated. Available tests for human health concerns are very poor for predicting health effects except for bioconcentratable substances. And such tests are not likely to be useful for substances that concentrate in sediments.

Many of us in the U.S. feel that the limitation of toxicity in effluents will move us a giant step forward in a cost-effective way to further reduce the emission of toxicity where it is likely to be of most concern. We see this limitation as a supplement to and not a replacement for more conventional control requirements.

In our research efforts to evaluate the validity of effluent toxicity tests we have completed studies in many geographic areas of the country and many types of receiving waters and effluents. Our objective has been to measure effluent toxicity and based on these toxicity tests predict what concentrations of effluent should cause impact in the stream. By doing dye injection studies we calculate the ambient effluent concentrations and then establish stations for biological sampling where various effluent concentrations occur. The measured impact is compared to the predictions to see if the effluent toxicity tests were valid predictors.

We also devised what we call an ambient toxicity test. In these tests we take water samples from the receiving water and measure the toxicity. The results of these tests can be compared directly to the biological field data and also to the effluent tests if the effluent concentration is known at the location where the sample is taken. Thus a three-way comparison can be made to see how good the agreement may be. These ambient tests were originally devised to measure the persistence (or die-away) of toxicity. They have proven to be extremely valuable tests for a variety of purposes including as a broad survey tool to identify segments of water bodies having toxicity, to locate new point sources of toxicity, to identify upstream water quality problems and to verify effluent toxicity test results. This test was made possible as a result of the development of short, chronic tests that can be performed with approximately the same effort as for a 96 hour acute fish toxicity test. Ceriodaphnia dubia/affinis is one test organism and a full life cycle test can be completed in 7 days elapsed time. The working time is about 6 hours for the entire test if 5 treatments and a control are used. Thus for ambient testing, if stations are placed 16 kilometers apart, 160-200 kilometers of stream can be assessed for toxicity with less than 2 person-days of effort not including sample collection time.

We have also used a 7-day larval growth fish test as a second estimate of toxicity. We use the fathead minnow but many species of fish will work as well. This test is also 7 days and we routinely get 4-6 fold weight increase in 7 days which is ample to weigh accurately. This test has proven to be very sensitive as well. The data from the studies we have completed clearly show the need to do at least two species. Frequently one or the other species has been insensitive to the toxicity displayed by the second species.

Figure 1 shows the correlation between the ambient toxicity (young/female) and benthic community loss, diversity and number of species. Figure 2 shows a similar correlation for algal diversity. There was poor correlation with the fish community because the fish were much less sensitive to one of the three effluents, than were the Ceriodaphnia. Figures 3 and 4 show plots comparing ambient toxicity as measured in the laboratory tests with the number of species and taxa of benthos and fish for two successive years.

Considering that the community measurements reflect the last several months of exposure variation whereas the toxicity is based on 7 days of sampling in one year and one day in the other year and also considering the effect of habitat differences on the community measurements, we feel the results are in excellent agreement. We are completing the reports for studies finished at other locations and the predictability has been comparable at these sites. In the studies where dye injection was done and effluent concentrations are known, the effluent dilution tests were also accurate predictors of instream impact.

We feel that all of our studies clearly support the validity of effluent and ambient tests for predicting community impact. Of course the more species that are tested the better the prediction will be. Several authors have examined the question of the benefit gained by testing additional species. Blanch (1984) found that some species of algae are more useful as representatives of species groups than others. He agrees that a few well chosen test species can provide good estimates of species sensitivity with reasonable confidence. We feel that the good correlations we have found between test species and impact on the community suggests that the Ceriodaphnia and fathead are a good choice for effluent testing. There will always be cases in which impact will be underestimated if only a small number of species are tested. No species is always the most sensitive. However, practicality limits testing to a small number of species. An additional amount of protection is provided if, as is the case in the U.S.A., limits are based on a critical, very low flow. If this is done then the duration during which the maximum exposure occurs is relatively short.

Most substances found in effluents are not as persistent as the chemicals that have been so problematical such as DDT and PCBs. To the extent that the substances in effluents decay, toxicity decreases and the area of exposure is smaller. Most of the effluent toxicity testing in the U.S. has not considered factors that diminish the real world exposure, such as persistence and application of limits that are protective at low flow, but have measured only toxicity. In order for impact to be underestimated several events must occur together otherwise there is enough safety margin inherent in the limits and the way they are applied to protect.

In the 8-10 sites we have studied in our recent effluent testing program, some surprises have been encountered. In no case have we seen synergism, only rarely have we found additivity and negative interaction has been common. Antagonistic effects have played a very important role in altering toxicity. One reason these effects have not been more often reported may be because most effluent testing has been done in relatively pollutant-free water for dilution. Since our objective has been to predict impact below outfalls, we have used dilution from immediately upstream even if it contained high concentrations of other effluents. Failure to use such dilution water is likely to over-estimate toxicity and cause one to conclude that effluent tests are not good predictors of impact in the receiving water.

Based on our experiences during the past 3 years, we believe knowledge is far enough advanced to make toxicity control practical and a better alternative in some instances than previously used practices. We see toxicity control as a supplement to and not a replacement for single chemical limitations in permits. We feel that such limitations are necessary in addition to single chemical ones because of the large number of chemicals in use and the slow progress in developing limits for single chemicals.

There is a pressing need for methods to measure the persistence of toxicity at an economical cost in order to avoid excessive limitations based only on toxicity. There is also a pressing need for a better comprehension of fluctuating exposure effects. Since effluent characteristics vary over time and dramatically so for some, the conventional constant exposure data customarily developed may not be applicable in some instances.

Finally, the way in which limits are translated into control requirements has an immense bearing on how stringent or protective the limits turn out to be. For instance if no effect levels are applied to a very low flow that happens only infrequently, the maximum permissible concentration will seldom occur. The regulatory strategy must be considered as a part of the limitations just as much as the way in which the toxicity tests are conducted and the data are interpreted.

Reference

Blanch, H. 1984. Species dependent variation among aquatic organisms in their sensitivity to chemicals. Ecological Bulletins 9185/36: 19, Stockholm.

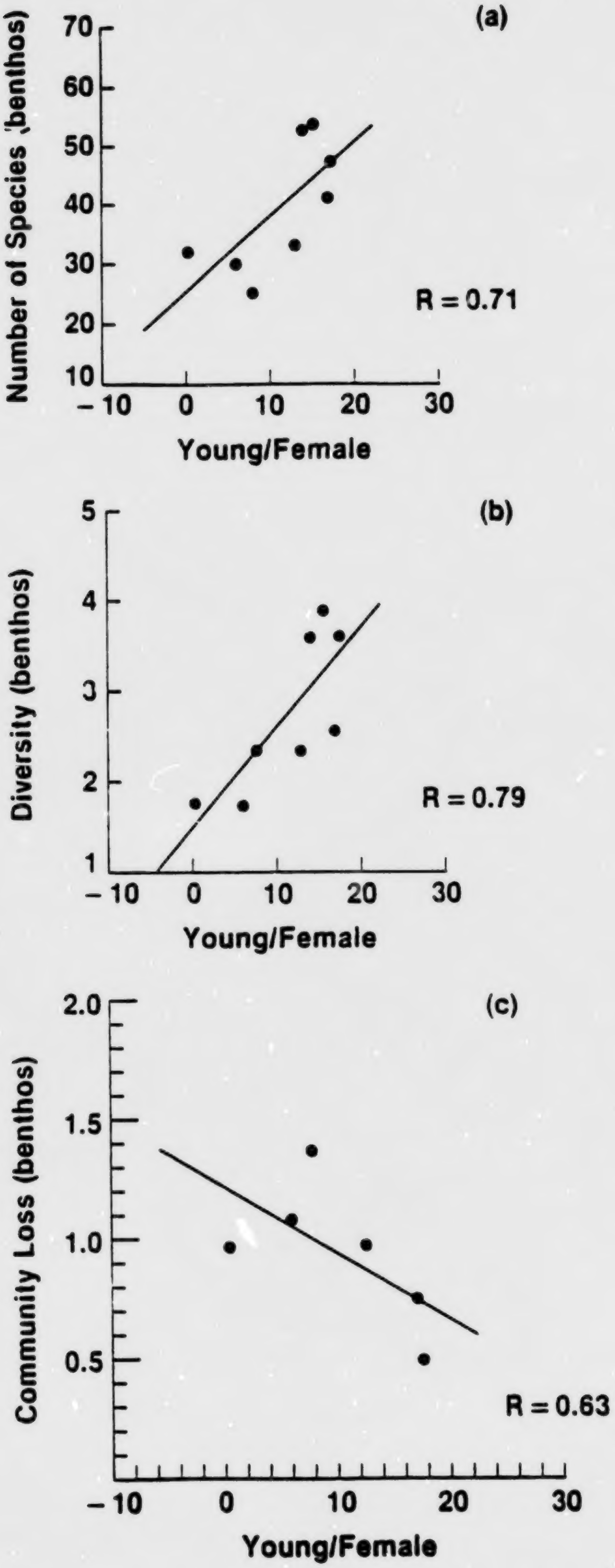

Figure 1. Correlation of *Ceriodaphnia* young per female with benthic parameters from eight stations in the Ottawa River, Lima, Ohio, 1982.

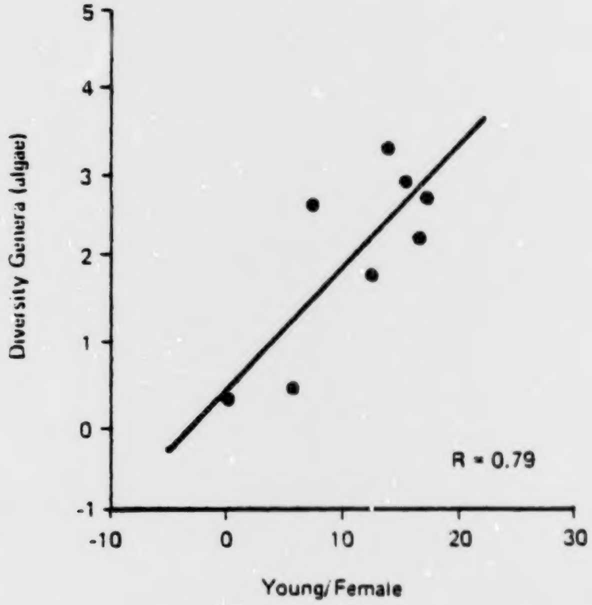

Figure 2. Correlation of *Ceriodaphnia* young per female and algal diversity at eight stations in the Ottawa River, Lima, Ohio, 1982.

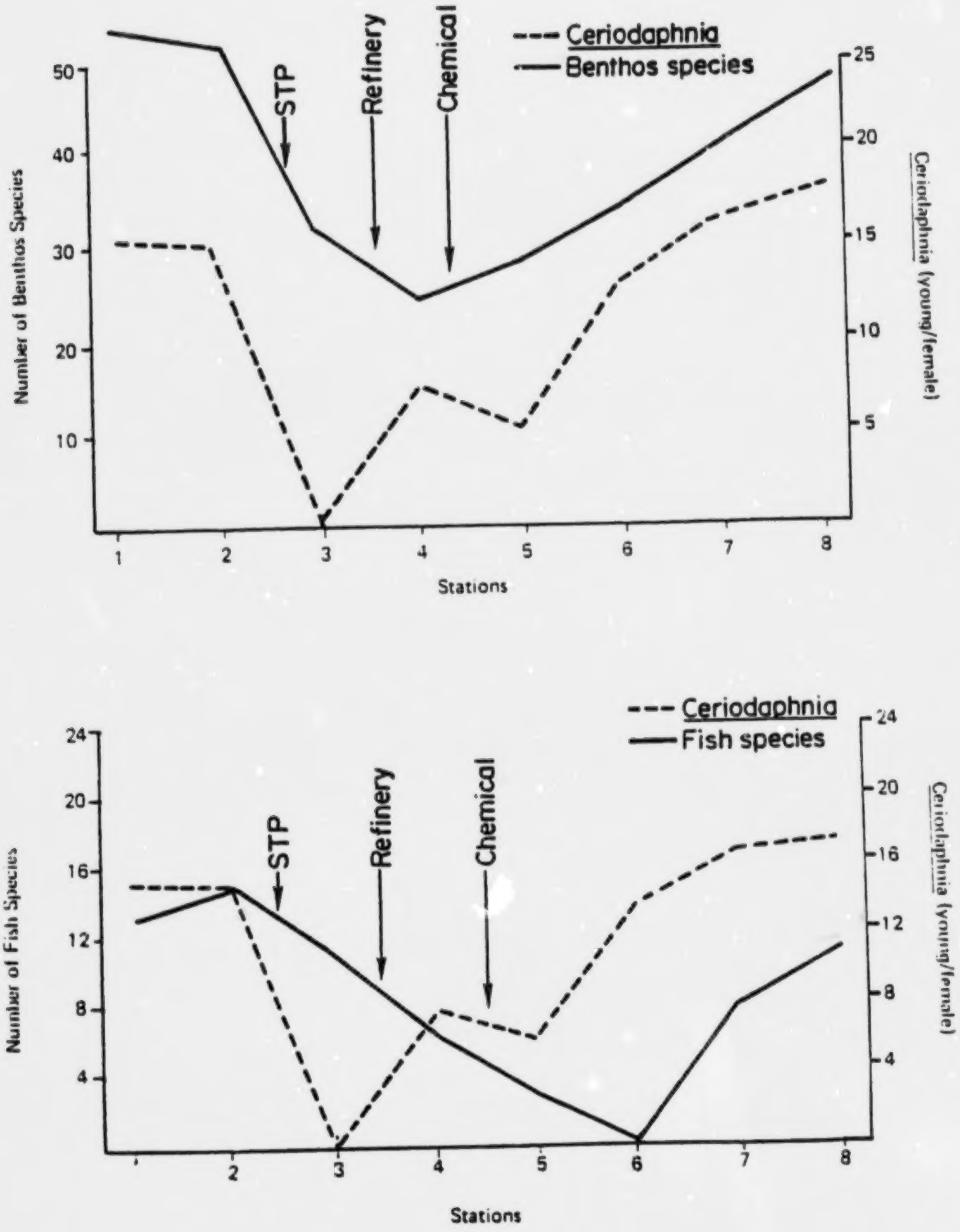

Figure 3. Ambient toxicity correlation between *Ceriodaphnia* young per female and ecological survey data for 1982.

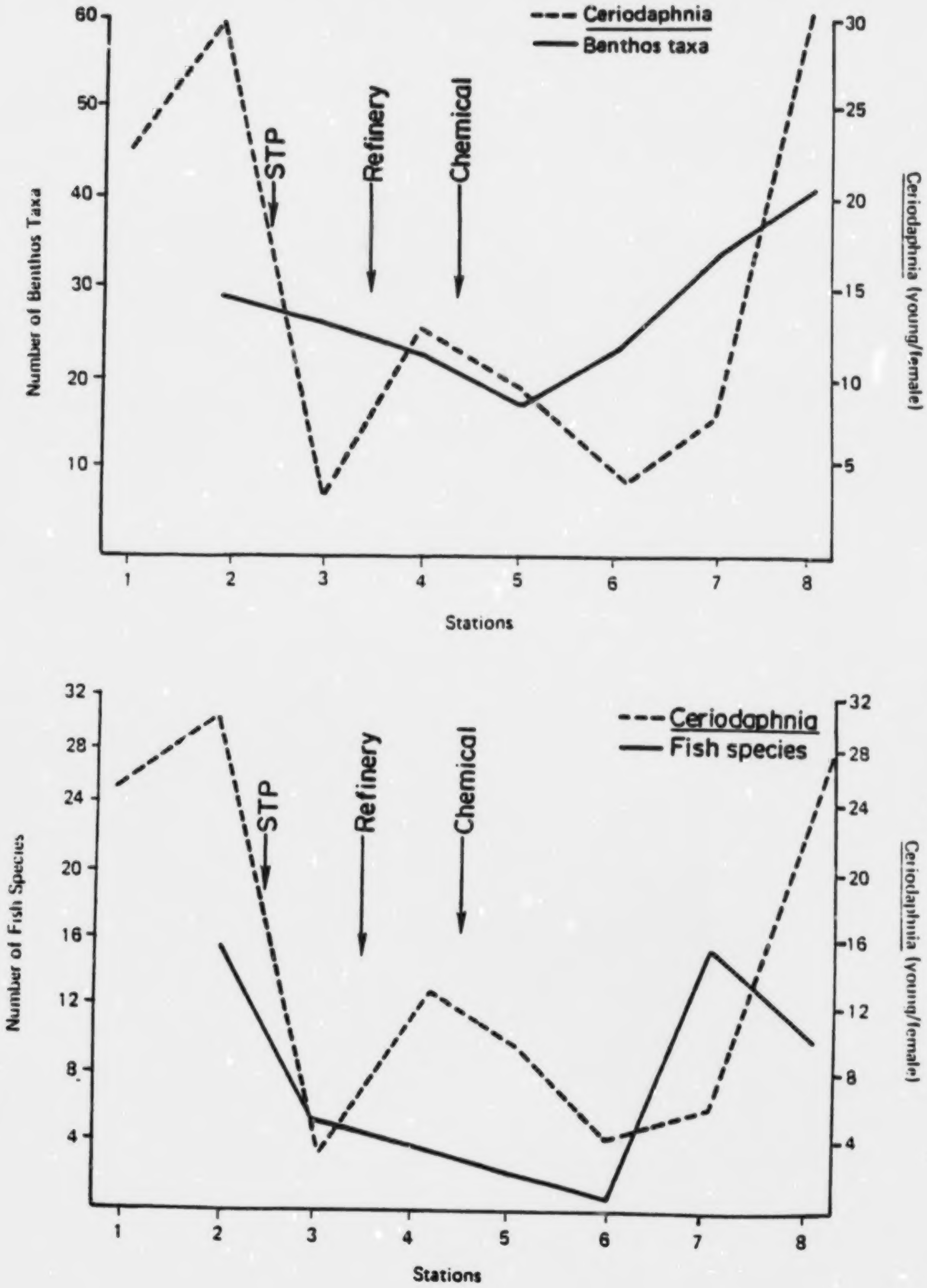

Figure 4. Ambient toxicity correlation between *Ceriodaphnia* young per female and ecological survey data for 1983.

THE OECD GUIDELINES FOR TESTING OF CHEMICALS

THEIR RELEVANCE FOR BIOLOGICAL TESTING OF EFFLUENTS

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The OECD Guidelines for Testing of Chemicals are the product of a intensive international programme which began in 1977. To date, seventy guidelines have been adopted by the Council of OECD. They reflect the consensus views of experts in many Member countries with respect to best available and cost-effective methods for testing of chemicals in the laboratory. A mechanism to improve the guidelines on the basis of the experience gained with them and the advancement of the state-of-the-art is provided through the Updating Programme. The list of the OECD Guidelines is appended.

Some guidelines concern the measurement of the intrinsic physical chemical properties of chemicals. These can be used to assess the likelihood of the occurence of a chemical in the environment and to develop exposure scenarios. Most guidelines, however, are aimed at the identification and the quantification of an effect observed in living organisms following exposure to a chemical under controlled conditions. The latter are relevant to the subject of the Workshop in-so-far as it will deal with biological testing approaches.

In the area of aquatic ecotoxicology the following guidelines may be particularly relevant:

201, "Alga, Growth Inhibition Test";
202, "Daphnia sp, Acute Immobilisation Test and Reproduction Test";
203, "Fish, Acute Toxicity Test";
204, "Fish, Prolonged Toxicity Study: 14 day study"; and
209, "Activated Sludge, Respiration Inhibition Test".

These guidelines have been developed at ad hoc meetings of experts on ecotoxicology and then finalized after several commenting rounds on successive drafts. A most important achievement was that the exports agreed on the animal species they recommended for these tests. Their exist national preferences for a particular species because of the experience already available with the species. It was recognized however that the comparability of results at the international level is helped by selecting a few species instead of having open-ended lists. Hopefully the species already selected in the chemicals guidelines exercise can be retained for the OECD Project on biomonitoring of effluents and for the proposed case studies.

The collection of OECD Guidelines contains a large section on degradation and accumulation. The methods used in these guidelines are derived from those used in the assessment of water quality. Therefore they are well known to the attendants of the Workshop. They deserve probably some attention in-so-far as they draw together experience at the international level.

The guidelines to measure biodegradability consider a parameter characteristic of the test system like oxygen consumption, CO_2 formation and loss of dissolved organic carbon. They do not call for application of chemical-specific analytical methods and are therefore readily applicable to the testing of whole effluents. In the tests for "ready biodegradability" pass levels have been agreed to. If the percentage biodegradation measured exceeds the pass level it is assumed that the substrate tested is ultimately biodegradable under aerobic environmental conditions. This concept may, however, have to be investigated with respect to its applicability to the testing a mixture of unknown composition. Essential characteristics of these guidelines are given in the table below.

TABLE: GUIDELINES FOR READY BIODEGRADABILITY

	parameter measured	duration of test	pass * levels
301A "Modified AFNOR Test"	dissolved organic carbon	28 days	70% loss of DOC **
301B "Modified Sturm Test"	CO_2 released	28 days	60% of theoretical amount
301C "Modified MITI Test"	oxygen consumption	28 days	60% of COD **
301D "Closed Bottle Test"	oxygen consumption	28 days	60% of COD **
301E "Modified OECD Screening Test"	dissolved organic carbon	28 days	70% loss of DOC

* Corresponds to "readily biodegradable"

** DOC = dissolved organic carbon, COD = chemical oxygen demand

In cases where the pass level is not attained, further scrutiny of the persistence may be called for and to this end a further set of guidelines for "inherent" biodegradability has been developed:

302A, "Modified SCAS Test"

302B, "Modified Zahn-Wellens Test" (being updated at present)

302C, "Modified MITI Test (II)"

Inhibition of biodegradation due to toxic effects on the micro-organisms can be evaluated with guideline 209 of the section on ecotoxicology already mentioned.

Attention need probably not be drawn at this stage to the many OECD Guidelines dealing with short and long term toxicology, using rodents and terrestrial animals as test organisms. The testing for mutagenic potential is possibly more in the scope of a biomonitoring project. The current thinking in the area is that a first screening can be based on a reverse mutation assay in bacteria, indicating base-pair or frameshift mutations, eventually complemented by an assay for chromosomal damage in _in vitro_ mammalian cell cultures. The relevant OECD Guidelines would be:

471 _Salmonella Typhimurium_, Reverse Mutation Assay

472 _Escherichia coli_, Reverse Mutation Assay

473 _In vitro_ Mammalian Cytogenic Test

The determination of an end point in a single test organism exposed to a specific chemical may be a relatively easy task. The integration of the various end points in a hazard evaluation is more complicated. Gauging through laboratory tests an environmental situation which results from the interaction of a complex mixture with a complex ecosystem is even more difficult. Both exercises have however the common pre-requisite that they be based on reliable experimental methods.

The international acceptance of the OECD Guidelines for Testing of Chemicals and the increasing amount of testing which is done in the context of notification of new chemicals lead to rapidly increasing experience with the laboratory methods covered in the Guidelines. It is hoped that this experience will prove to be an important aid in the methodological component of the biomonitoring project.

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* Updated guideline, adopted 7th June 1984
** Updated guideline, adopted 4th April 1984

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A COMPARATIVE STUDY TO IDENTIFY THE MOST CONVENIENT BIOTESTS FOR A GIVEN SITUATION AND DECISION

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ABSTRACT

The aim of this paper is to identify the most suitable biotests to establish a scientific basis for making decisions concerning environmental protection in relation to various situations.

Biotests may be applied to several environmental problems, the most common being: 1. to assess the water quality of a river and its effluents; 2. to monitor a polluted water course and its effluents continuously; 3. to know the distribution of an effluent in the receiving water body and its effects on the biota; 4. to evaluate the effectiveness of a waste water treatment plant and/or any action to reduce the pollution loading to the water course; 5. to screen new substances according to their potential hazard and to compare the harmful effects of traditional pollutants; 6. to predict the effects of one or more pollutants on the natural community.

For each of these problems the most suitable biotests are suggested and the advantages and the disadvantages of each biotest are discussed. From this discussion it can clearly be seen which biotests need more study and which may be applied without additional research.

The aim of this paper is to identify the most suitable biotests in establishing a scientific basis for taking decisions concerning protection of the environment in relation to various situations. The advantages and disadvantages of each biotest are discussed and the need for research to improve the testing methods is stressed.

The various decisions concerning the environment must be based on the knowledge of the actual situation, that is, a picture of the environment obtained from the knowledge of the physical, chemical and biological properties of the environment and the modifications due to the activities of man. These studies, which are the task of the ecologist, require a specialized staff and generally produce, in a relatively long time, results which may be suitable only in part for taking decisions.

Because in any decision time and cost must be considered, various biotests have been developed to obtain by simple methods and in a relatively short time the information of use to the decision maker.

Any biotest is based on the very simple principle that the organisms react to the environmental conditions and, consequently, these reactions are a good index of the state of the environment and its variations produced by natural stresses and/or human influence. The reactions may be measured at different levels: community, population, individual, cell and tissue, biochemical processes, etc. In a strict sense, the bio-indicator is a biological reaction which is particularly suitable for estimating the potential hazard to the whole community. Consequently, an increase of the mortality of a species sensitive to the pollutants, alterations of the physiology or the behaviour of a species, and the modification of the enzymatic activity may be considered biological indicators /1/. More commonly, a biological indicator is a taxon (species, genus, family) sensitive to one or more pollutants.

Some biotests may be applied in the field, other in the laboratory and other in semi-natural conditions. For the biotests used in the field, it is essential to choose the "critical period" of the year and the "critical area" in which to perform them. For example, in a river, during the flood period, the concentration of toxic substances will be a minimum, conversely, during the low waters the highest concentrations will be measured. Upstream of the effluent the pollutants will be absent, while downstream the concentration of the toxic substance will decrease as the distance from the effluent increases. It is obvious that biotests cannot replace chemical analyses of the environment, because the type of information obtained with biotests is substantially different from that produced by chemical analyses. Indeed, the biotest reacts to the global situation, which is the result of the ecological factors (including pollution), whereas with chemical analyses we identify the pollutants and their concentration, limited to the time of the sampling, but not their effects on the biota.

Biotests are applied to several problems, the most common being: 1. to assess the water quality of a water course and its effluents; 2. to monitor a polluted river and its effluents continuously; 3. to know the distribution of an effluent in the receiving water body and its effects on the biota; 4. to evaluate the effectiveness of a treatment plant and/or any action to reduce the pollution loading to a water course; 5. to screen new substances according to their potential hazard and to compare the harmful effects of traditional pollutants; 6. to predict the effects of one or more pollutants on the natural community.

1. TO ASSESS THE WATER QUALITY OF A WATER COURSE AND ITS EFFLUENTS

The first approach to classifying the water quality by biological indicators dates back to the middle of the last century /2/. Since that time about fifty biological methods for water quality assessment have been proposed. Reviews of these methods have been made by Slàdecek /3/, Persoone and De Pauw /4/, Illies and Schmitz /5/ and others.

Three intercalibration campaigns sponsored by the Commission of the European Communities (CEC) showed about 20 different methods for assessing the water quality commonly used in Europe /6/. These biotests are based on the presence (or absence) of species (or groups of species) well known for their resistance or their sensitivity to pollutants, respectively; for example /7,8,9,10/. An example of these methods is the subdivision of a water course into zones according to the characteristics of its fish population /11/, its benthic invertebrates /12,13/, or its micro-organisms /14,15,16,17,18,19, 20,21,22 and 23/.

"Biotic indices" have been developed on the basis of the characteristics of the mesobenthic invertebrate association (currently called "macroinvertebrates") to classify the water quality; for example "Trent Biotic Index" /24/, "Extended Biotic Index" /25/, "Indice Biotique" /26/, "Belgian Biotic Index Method" /27/ and "Score System" /28/.

These systems essentially consist of a numerical scale in which each number indicates a certain structure of the association corresponding to a probable level of degradation of the water quality. With the same aim the "Saprobic scale" has been developed which is based on the association of micro-organisms (e.g. bacteria, fungia, protozoa, algae).

These methods have been used to build up maps indicating the different water quality levels of the rivers of a given area by different colours (e.g. Baden-Württenberg, Austria). In several cases micro- and macrofauna and microflora colonizing artificial substrata has been used to determine the pollution level of water courses /29/.

One of the most common effects of pollution, both domestic and industrial, is the modification of the community structure. The description of the community structure and its modifications may be quantitatively described by the several "diversity indices" developed. This method is laborious and requires specialized personnel and the results obtained cannot always be explained easily. Consequently, up to now the "diversity index" is an interesting research method, but not very useful for practical uses /30,31/.

These methods, developed for streams and rivers polluted by domestic waste waters, were then applied to industrial pollution. From them valuable information may be obtained, especially if the state of the water course (or the effluent) is known both before and after the pollution discharge. Some methods /32/ combine chemical monitoring with biological monitoring. The results obtained seem to be satisfactory.

2. TO MONITOR A POLLUTED RIVER AND ITS EFFLUENTS CONTINUOUSLY

To do this we need a method giving a clear answer in a very short time. With this aim two different tests may be adopted: a) to detect the effects (generally mortality) on animals kept in cages submerged in the water course and/or in its effluents. The animals (e.g. fish, molluscs) must be collected from environments similar to that to be tested, but not polluted or with a minimal level of pollution; b) to detect the effects of pollution on animals (e.g. fish, molluscs) kept in aquaria which are fed by the water course to be tested or by the water of its effluents, for example /33,34/. To simulate the effects of an effluent a series of aquaria may be fed by different proportions of effluent water and river water.

These tests, in addition to the continuous monitoring of the water course and its effluents, may be used as an "alarm test", i.e. in the case that a pollution wave is transferred along the river or a large amount of toxic material is illegally discharged into an effluent.

3. TO KNOW THE DISTRIBUTION OF AN EFFLUENT IN THE RECEIVING WATER BODY AND ITS EFFECTS ON THE BIOTA

The use of tracers (e.g. fluorescein) may give general information on the distribution pattern of the effluent water into the receiving water body (e.g. river, lake, coastal waters). Because the distribution of the toxic substances present in the effluent may not be identical to that of the effluent water, water samples collected in the critical points chosen on the basis of the tracer distribution must be chemically analysed.

By this method the distribution map of the toxic substances in the receiving waters may be built up, but no information on the biological effects can be obtained. With the use of bioindicators the different levels of hazard due to the effluent may be detected in different areas of the receiving water body, that is, the extent and the severity of the effluent pollution.

Two methods may be applied to do this. One consists in measuring the mortality after a short time of a group of animals (e.g. fish) kept in cages submerged at different points of the polluted and less polluted areas. The other method uses artificial substrata placed in different points of the polluted and control areas. From the type of organisms colonizing the substrata (fouling) the level of pollution may be determined. This method requires specialized personnel and produces after a relatively long time information which is variable with the season and environmental conditions.

As we have noted above, chemical analysis of the water reveals the environmental situation at the instant of sampling as opposed to the reaction of the organism which is the cumulative result of preceeding and present levels of pollution. If this information is needed, resistant indicators to the pollutants present in the environment must be used as opposed to the sensitive bioindicators chosen for the "biotic indices".

Of these resistant organisms the species accumulating pollutants are suitable for mapping the most polluted zones and identifying the toxics responsable for the pollution. These organisms, because of their high accumulation capacity may be called "bioaccumulators" instead of bioindicators. These organisms are collected from different zones of the area receiving the effluents and are chemically analysed.

The filter feeding bivalves (e.g. Dreissena, Unio, Anodonta, Mytilus) concentrate several of the most dangerous pollutants (e.g. transuranic elements, radioisotopes, halogenated hydrocarbons, hydrocarbons, heavy metals) from the water by factors of even several thousand over the water concentration. They are widespread, sedentary and, generally, sufficiently abundant that frequent sampling will have no appreciable effect on the populations. Because of these characteristics and their extreme resistance to pollutants, bivalves are ideal "sentinels" in evaluating the importance and extent of pollution; for example /35/.

4. TO EVALUATE THE EFFECTIVENESS OF A TREATMENT PLANT AND/OR ANY ACTION TO REDUCE THE POLLUTION LOADING TO A WATER COURSE

The same methods used to assess the water quality of a river and its effluents (see 1) may be used for this. It is obvious that the testing must be done before and after the actions to reduce the pollutant loading. The testing must be repeated in different seasons for a long period of time after the beginning of the water treatment in order to distinguish the effects of the treatment from those due to seasonal fluctuations.

5. TO SCREEN NEW SUBSTANCES ACCORDING TO THEIR POTENTIAL HAZARD AND TO COMPARE THE HARMFUL EFFECTS OF TRADITIONAL POLLUTANTS

Toxicity tests are not only desirable, but also sometimes required before a product can be marketed. To carry out these tests the method commonly adopted consists in laboratory experiments in which the effects of the new substances, suspected to be dangerous, and traditional pollutants are recorded. The organisms used in laboratory experiments represent "biological sensors" of the toxicity of any pollutant and for this reason they may be used to classify the new and traditional substances in relation to their level of toxicity. In addition, information on bioaccumulation and biodegradation rates for the substances considered may be obtained from these experiments. A valid test must have a high level of sensitivity, the results must be reproducible and the techniques used should be very simple and economical. The situation today is that an enormous amount of information has been obtained from laboratory experiments, but the discrepancy of the results is large and it is not unusual to find differences in values in the range of 10^3 in different studies on the effects of the same pollutant on the same species. The reasons for this discrepancy are numerous and include: a) different laboratory conditions during the period of acclimatization and experimentation; b) different physiological and genetic characteristics of the organisms studied; and c) differences in the handling of the data and presentation of the results, e.g. /1/.

Experiments are carried out in batches or with a continuous flow of the solution to be tested. If the flow of the solution is continuous, some causes of error may be abolished or minimized; for example, the adsorption of the pollutant by the container, the reduction of dissolved oxygen due to the respiration of the organisms, the accumulation of catabolic products and variations in pH value. If it is impossible to have a continuous flow of the solution, it should be renewed at least once a day. This method is inexpensive and can be carried out easily, but the organisms tested may be disturbed. The most commonly used response of a species to the pollutant is the mortality (LC-50); measured after a prescribed time (e.g. 24, 48, 96 hours), of a group of organisms exposed to the pollutant (or a mixture of pollutants). Another method is to measure the time it takes for 50% of the organisms tested to die (ST-50). Mortality is not a very sensitive response but it has the advantage of being easy to record; for example /36/. The behaviour of the organism in contact with the pollutant provides an instantaneous and highly sensitive answer, for example /37,38/. Other types of response can be evaluated only over a relatively long time; for example, individual growth-rate, variations of the population density (used for culture of micro-organisms), reproductive characteristics (fecundity, fertility), photosynthetic-rate, oxygen consumpion, delay in embryonic development and percentage of abnormal embryos. Responses may be divided into two groups: those that deal with acute effects (short-term effects, that is over hours or a few days) and those that involve chronic effects (long-term ones, usually over times longer than a week). The decision to measure acute or chronic effects depends on the aim of the test and is related to the concentration and toxicity of the pollutant, the sensitivity of the organism and the type of response. In short-term tests it is easier to maintain the environmental conditions constant throughout the experiment but, generally, they provide less adequate information on the actual toxicity of the pollutants.

Short-term tests are adopted to ascertain the mutagenic and carcinogenic properties of the substances. Long-term tests require more sophisticated techniques, but they provide highly reliable information.

It should be stressed that it is not correct to use short-term tests with high concentrations of pollutants to reach conclusions about long-term effects at low concentrations /39/.

A biotest on one species cannot give reliable information about the effects of any pollutant at the community level, because of the different sensitivity of the different taxonomic groups to the same pollutant. To do this selected species representing the most important taxonomic groups of the community must be tested.

6. TO PREDICT THE EFFECTS OF ONE OR MORE POLLUTANTS ON THE NATURAL COMMUNITY

In laboratory experiments each environmental factor is commonly maintained constant. If more than one species is used in the same container (as occurs with a "balanced aquarium") negative interspecific relationships (e.g. predation, parasitism) must obviously be prevented and intraspecific competition must also be abolished or attenuated. Conversely, in natural ecosystems the environmental factors change continuously and there are interrelationships between different species and between the species and the abiotic components of the ecosystem. In addition, the pollutant is influenced by the changing environmental factors which modify the toxicity and the availability of the pollutant to the organism. In most of the experiments a single or few pollutants are tested; whereas in the natural environment, organisms are exposed simultaneously to several pollutants and, therefore, they react to the entire environmental situation. For these reasons the laboratory conditions are very artificial and, hence, it is difficult to predict the effects of any type of pollution in the natural communities using results obtained from laboratory studies.

The studies carried out in the natural environment are essential, but because of the complexity of the ecosystems, and particularly of those under stress, it is rather difficult to draw practical conclusions. We need an experimental plan representing a compromise between laboratory experiments and studies in the natural environment, i.e. experiments carried out under semi-natural conditions, which allow us to follow the effects of a single pollutant (or a mixture of pollutants) in a representative portion of the community. In addition, by this technique, the biological effects produced by a reduction (or increase) of the pollutant loading to the natural environment may be predicted. This information is very useful for planning the recovery of polluted environments and protecting those which are not polluted. The methods to achieve these goals may be grouped into two types: "enclosures" and "outdoor experimental channels".

The "enclosure" method consists in isolating, by plastic sheets, a portion of the environment to be studied /40,41,42,43/. The main advantages of this technique, when compared to laboratory experiments, are the following: a) the structure of the community and the population density of each species are those of the natural populations and community; b) the variations of some parameters (e.g. light, temperature) are identical to those of the environment considered and c) at the beginning of the experiment the physical, chemical and biological characteristics are the same inside and outside the "enclosure". Unfortunately, there are also some disadvantages: for example the "wall effect", the prevention of horizontal water transport, the reduction of turbulence and the abolition of nutrient loading from the watershed. The pollutant(s) is introduced into the enclosure, generally, at the beginning of the experiment, but in a few cases it has been continuously added to the enclosure during the experiment /44/. The results obtained by the "enclosure" method, adopted for pesticides, heavy metals and hydrocarbons in fresh and marine waters, are promising.

The method of the outdoor experimental channel consists of one (or more) artificial channels, made of plastic material or asbestos cement, in which the water to be tested flows. The bottom may be covered by pebbles, sand or mud or by a mixture of these materials.
The water is diverted from a river or from treated or untreated effluents and discharged into the channel at a prefixed flowrate. For certain aims known amounts of domestic sewage or industrial pollutant(s) are added with a doser system to the channel. A multichannel system offers the possibility to study the biological effects of different types of waters under a uniform set of climatic variations. In the channel some species, generally fish, are introduced to evaluate the resistance and bioaccumulation-rate of the pollutant tested /45/. In other experiments no organism is introduced and the effects of the pollutants on bacteria, algae and macroinvertebrates colonizing the artificial channel are recorded /46/.

DISCUSSION AND CONCLUSIONS

Various biological methods for monitoring and controlling the watercourses and the domestic and industrial effluents have been developed. It is evident that there is no single best method, but for each type of situation and decision some methods are more suitable than others. The ecotoxicological studies produce results which represent the basis for developing the biotests, but the concept of biological effect differs substantially from that of biotest. Indeed, the research on the biological effects of the pollutants may have no practical aim, whereas the study of the biotest is necessarily oriented towards a well defined practical goal, because it applies only to those biological reactions that are considered suitable for estimating the potential effects of pollution on the community.

For the same type of biotest there are numerous modifications and it is difficult to say which are the best. For example: in laboratory tests the characteristics of the dilution water, the species and the biological responses vary according to the researcher and the region in which the test is performed. This variability produces very rich information on the potential sensitivity of the biota to a given pollutant. On the other hand, the advantages of working with standard material and adopting standardized methods are obvious. Standard material means individuals of the same species, the same age and sex, genetically and physiologically homogeneous. As a consequence, it is advisable to have banks of organisms reared in standard conditions and in sufficient quantity to be distributed to all the laboratories needing the same species. Standardization of methods is often difficult to obtain for various reasons, some valid, some less so. When it is not possible to standardize the methods, these may be harmonized, that is, a single scale can be developed to compare the results obtained with different techniques. Harmonization is particularly useful when a watercourse and its effluents must be monitored and controlled by different laboratories of the same country or, in the case of an international watercourse, such as the Rhine, by laboratories which have adopted methods varying from one country to another. Unfortunately, it is not easy to harmonize different techniques. In this manuscript I have classified the different biotests according to different situations and decisions, but more techniques may be successfully applied for the same decision. For example, to predict the biological effects produced by the reduction of the pollutant loading of an effluent inflow into a river, more methods may be applied, for example to compare the values of a biotic index upstream and downstream of the discharge of an effluent, to carry out laboratory experiments with a series of concentrations of the effluent water or to simulate the biological effects in the river with outdoor artificial channels fed with different concentrations of the effluent water.

In conclusion, if it is important to carry out studies on biotests to improve their reproducibility and sensitivity, it is far more urgent to develop standardized biotests to be applied at least when the same level of control must be exerted on different effluents and waterbodies; for example, on international rivers and on effluents of the same country. Local organisms may replace the standardized material if intercalibration between different organisms is done, that is, to measure the response to the same pollutant of relative species living in different areas. Because the biotest techniques are based on ecotoxicological research, this must be encouraged. In addition, we need more research on the "enclosure" and outdoor artificial channels in order to obtain results more suitable for application to the natural situation.

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USE OF BIOMONITORING IN WATER QUALITY REGULATORY DECISIONS

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Abstract

Based on the present uncertainties in pollution control it is argued that biological monitoring seems indispensible as an additional tool to signal, predict and control hazardous chemical water pollution. However, as in most countries, in The Netherlands biomonitoring activities are fragmentary. Experience learns that the present regulatory framework is repressive to research efforts, whereas these efforts are necessary to provide the scientific information to influence the regulatory policy.

Application of biological assessment and monitoring would be enhanced if it were possible to establish an integrated ecotoxicological approach in a manner similar to the step-wise predictive hazard evaluation system for chemicals. For biomonitoring, however, such construction will be more complex since several objectives have to be fulfilled and different parties will be involved. Therefore it is recommended to limit the attention towards objectives that fit into the regulatory system and to focus on the development and completement of a tiered monitoring program.

As a first step in biomonitoring it is proposed to balance the efforts by waste dischargers and surface water management services and to suggest simple screening techniques. As to effluent control, this may hold the application to toxity bioassays according the the OECD-guidelines for Testing of Chemicals, to predict the adverse effects of the effluent before release. However, in some cases the use of automatic early warning systems will be indispensable. As to the management of surface waters the first step may be filled in by short-cut (geno)toxity techniques in which hazardous components in the water are concentrated before testing to speed up the response rate.

INTRODUCTION

Within the few last decades pollution of our environment has become a significant problem. Extremely large numbers of chemical substances have been produced and used, without knowledge of their environmental impact. As a result, on several occasions people have fallen victim to chemical water pollution. The subsequent growing public awareness of the need for protection has led to a number of standards and regulations in many countries of the world. At first these were chiefly aimed at protecting human health. According to the current view, however, the improvement or defence of the water quality should also be directed towards providing favourable conditions for regaining or maintaining as much as possible the natural variety of organisms and aquatic ecosystems [15].

These days, production, use and discharge of hazardous substances is generally governed by well-defined regulations aimed at the protection of both human health and ecosystems. However, questions arise whether these regulations indeed offer the protection envisaged and whether the present tools in the decision making processes are the most effective ones. If there is any doubt about these points, one may wonder whether the additional use of biomonitoring could play a role in improving the efficiency and effectiveness of water pollution control.

In this discussion paper an attempt is made to answer these questions and the need for implementing biomonitoring is emphasized. Finally a step-wise monitoring program is proposed and some methods are recommended to complete the basic level of biomonitoring.

BIOMONITORING

Definition of biomonitoring

Before going into the why's, when's and how's of biomonitoring, the term biomonitoring needs to be clearly defined to avoid the otherwise inevitable confusion. For the same reason Hawkes [19] distinguished the terms survey, surveillance and monitoring:

- survey : an exercise carried out to establish a pattern of spatial variation at one point in time.
- surveillance : the repeated and standardized measurement of a variable in order that a trend in time may be detected.
- monitoring : a surveillance carried out to determine trends in relation to predetermined standards.

Essentially these terms are given in chronological sequence in which environmental research usually struggles along, with monitoring as a possible endpoint. Although these definitions are correct as such, they give a more limited sense to biomonitoring: it requires a regular repeated investigation, in which a biological variable is measured according to standardized methods and in addition to which the obtained measuring results are compared to predetermined standards. In the scope of this workshop, however, it is reasonable not to be so very particular. For in this restricted sense biomonitoring could take place only after the determination that emergency situations do happen and are expected, after the development of scientific sound and ethical and economical acceptable standard methods, and after the establishment of scientific and political justifiable standards.

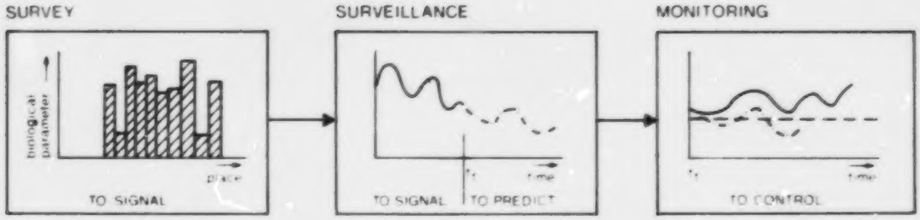
In practice this means that only a very few biological environmental research activities could be addressed to as biomonitoring. Taking into account the present developmental stage of biological monitoring, as well as the concern not to loose ourselves in generating data for the sake of biomonitoring, we prefer the more flexible definition according to Matthews et al [31]: the use of biological responses to evaluate changes in the environment (i.e. effluents and surface waters) with the intent to use this information in a quality control program. In this respect it is emphasized that the measurement of concentrations of environmental contaminants in biological materials as such is considered as chemical monitoring (e.g. the activities of the Joint Monitoring Programme).

Functions and application possibilities of biomonitoring

As shown in Fig. 1, biomonitoring could fulfill three different functions: to signal, to predict and to control. By measuring at different locations or in different waters spatial differences in water quality can be observed, whereas by regular measurements at one point in time temporal changes can be discerned. As to the latter, trends may be detected, on which base predictions can be made. In case the observed change, difference or prediction leads to action, biomonitoring could be used to induce that action (e.g. early warning systems) or to check the effectiveness of the action.
In Fig. 2 some application possibilities of biomonitoring in the aquatic environment are illustrated. Basically, there are three potential users of biomonitoring:

(a) Waste water dischargers

- to assess the toxicity of waste waters and to check on effluent limitations.
- to protect receiving system from damage.


SURVEY
SURVEILLANCE
MONITORING
biological parameter
place
TO SIGNAL
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TO PREDICT
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TO CONTROL

Fig.1 The three functions of biomonitoring: to signal, to predict and to control (modified after [20]).

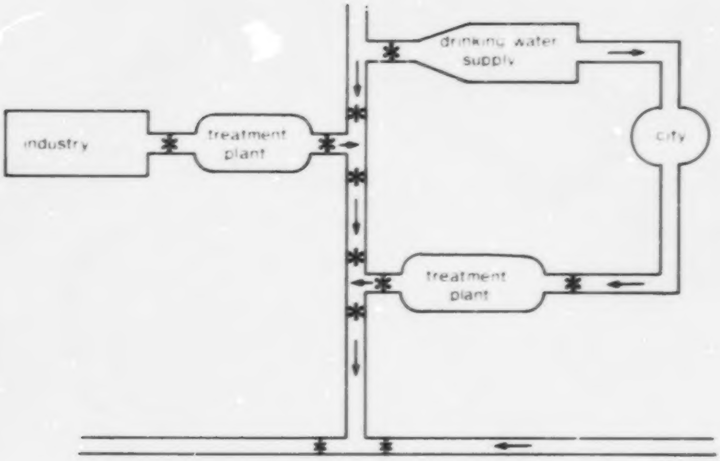
drinking water supply
industry
treatment plant
city
treatment plant

Fig.2 Application possibilities of biomonitoring in the aquatic environment by waste dischargers (domestic and industrial effluents, receiving waters), water management authorities (receiving waters) and water users (water inlets).

- to protect waste water treatment systems from disfunctioning.
- to determine whether further technological improvement in waste treatment have significant biological benefits.

(b) Regional and national water management authorities

- to establish biological water quality objectives and criteria in view of policy options as to restoration or enhancement of aquatic ecosystems.
- to trace down pollution sources (both point and non-point sources).
- to detect the development of unfavourable conditions for life.
- to control sanitation measures to estimate the degree of ecological improvement resulting from implementing improvements in waste discharges.
- to determine whether further legislative measures have significant biological effects.
- to validate or to improve the predictive capability of laboratory testing as to hazard assessment.

(c) Water users

- to detect accidental spills to prevent catastrophes as to specific uses of the water (fish farms, public water supplies).

This may be a lengthy list, but we should realise that it is likely that one biomonitoring system serves different purposes for the user. It is also anticipated that the results obtained with one system are of value to different interest groups. Furthermore, for some purposes the need for biomonitoring may be less in case of a proper control of the pollution sources.

The need for biomonitoring

From point of view of environmental protection

As to the first consideration there are indications that the environmental control of chemicals is still far from perfect in spite of the recent improvements. In case of existing chemical pollutants (30,000 - 40,000 within the EEC: [23]) we are confronted with the problem that even the most basic data of non-regulated chemicals are often lacking or not published. For example, of the thousands and thousands of organic substances present in the heavily polluted river Rhine, only 384 have been identified so far, whereas respectively merely for 53 and 37 percent of these substances basic mammalian and aquatic toxity data are available [25]. In the selection of priority pollutants production volumes, among others, are used as a criterion, whereas it is well known that relationships of production volumes and the actual environmental concentrations are often very complicated. In this respect it also may be of interest that the priority pollutants as selected by the EEC and IRC do not as yet include the most toxic compounds identified in river Rhine water (Fig. 3).

The problem of lacking data seems not applicable to new chemicals. According the EEC directive 79/831, resembling the Toxic Substances Control Act, the notifier has to provide the authorities with the information required. However, it should be realised that in practice the information on the ecotoxicological characteristics is provided only for chemicals which are produced in quantities of more than 100 tons per year and about 2 percent reach a production level of 1000 tons per year [49]. Thus, since the tonnage level is the trigger mechanism in the requirement for more information, only some basic data will be available for about 80 percent of the chemicals.

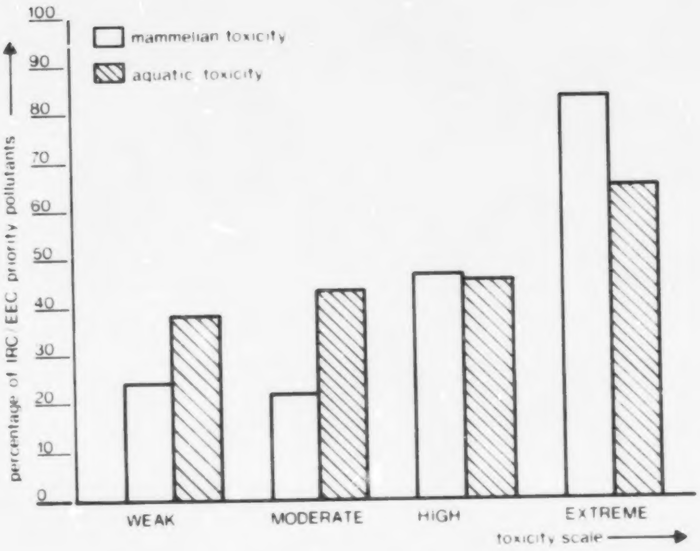

Fig.3 Percentage of IRC/EEC priority pollutants relative to the number of organic pollutants per toxicity class identified in river Rhine water (after [25]).

Chemical intermediates (by-products) are not accounted for. Besides, proof of hazardousness not necessarily results to restrictions upon production volume, use and release in the environment of a chemical, as this largely depends on the implementation of the EEC directive by laws of the member states. Hence, legislation deals with a selected, but relatively small number of chemicals and many environmental pollutants escape attention.

Another worrying aspect is that decision makers tend to endow the results of laboratory data with a significance and precision which is certainly not always merited [32]. Water quality criteria are in large part based on laboratory-derived fate and toxicity data and subsequently ruled by chemical parameters. Yet, in spite of several attempts, we have not been able to clarify the relationship of laboratory data to the field assessment of impact [4, 16]. Doubt about this relationship is growing since data are accumulating that (a) both the acute and chronic toxicity response to a substance differs widely among species [43, 45, 50]; (b) single species tests cannot provide the information about possible higher order effects [6], but even if they could, the problem of predicting effects from one (eco)system to another would remain [48]; and (c) the total concentration of a chemical, which is measured in dirty water may well not give the same response as the same concentration in clean water because of the differences in its biological availability [12, 32] or the presence of other chemical pollutants resulting in concerted action [24].

From this brief outline it can be concluded that, as formulated by Lubin and Moss [28], "the present environmental laws may be viewed as pragmatic attempts by the political system to deal with risk in a situation of incomplete data and scientific uncertainty". It is in this respect that many scientists share the feeling that the present system of environmental quality control is seriously hampered by the absence of biological control. Even more important, those who have to make decisions about the quality of life should be aware that public concern in pollution is solely centered on its final effects on living beings. This reality at least justifies the opinion that biological monitoring deserves more attention.

From an economic point of view

As to the economic aspects of biomonitoring a distinction should be made between cost-benefit and cost-effectiveness. Cost-benefit analyses are aimed at the determination of a monetary value for the benefits of achieving a goal, as in case of verifying biologically the results of regulatory measures in terms of defencing or improving the quality of life. In cost-effectiveness analyses an attempt is made to determine the less expensive way if the same goal can be achieved in different ways, in this case physico-chemical monitoring and biomonitoring.

Unfortunately reliable figures on both types of analyses concerning biomonitoring are generally not available. Benefits are difficult to estimate since life is not easily translated into monetary terms [19]. It involves risk evaluation and deals with the question "how safe is safe". It is therefore a fundamental and socio-political value judgement out of the scope of this workshop. As to the effectiveness of biomonitoring versus chemical monitoring, this definitively will depend on the specific purpose of monitoring and the available sound techniques which method will prefer. Taking into account the complexity of monitoring objectives and the variety of methods published as being useful, we are presently not able to draw firm conclusions.

Biomonitoring versus physico-chemical monitoring

It is well known that monitoring programmes for pollution detection and control have been oriented mortly towards physical and chemical methods. The reason that biomonitoring has fallen into disrepute is that it is considered as confirmatory to physico-chemical methods, but with a variety of insurmountable drawbacks. Therefore there is no harm to draw attention to the major limitations and advantages of biomonitoring relative to physico-chemical monitoring moreover [7, 18].

The major advantage of biomonitoring over physico-chemical testing is that living organisms will respond continuously to every possible substance or mixture of substances at some level, no matter what their characteristics may be. In contrast to this chemical monitoring depends upon knowing the concentration level of pollutants and their environmental impact. This leads to the conclusion that until one can identify these compounds with certainty and measure their presence in waste water and selected compartments of the environment, effective control is essentially impossible, especially since for most chemicals data on their environmental impact are lacking. With the increased complexity of industrial effluents this becomes even more difficult since it is technically nor economically feasible to measure all possible dangerous pollutants qualitatively and quantatively on a continuous base. Even so, the present stage of science does not allow to predict harmful effects from such data.

On the other hand the usefulness of biomonitoring is limited. Detected biological responses may be indicative of water quality, but in most cases they do not provide the information necessary to identify the specific cause of the observed changes or differences. For that purpose chemical analyses will often be necessary. It should be noted, however, that for some monitoring purposes such identification is of minor importance. Moreover, chemical analyses may very well fail to identify the causative pollutant(s). Another weakness is in interpreting biological monitoring data in terms of water quality acceptable on ecological or public health grounds. This problem can be solved by applying appropriate biomonitoring methods and the requirement of additional microbiological and specific chemical tests successively. Furthermore, biological data may be not comprehensible to decision makers. Processing of the basic data to provide information that is less hostile to data users could raise this disadvantage. Another drawback often thought to be related to biomonitoring is that it is more expensive and not as reliable as chemical monitoring, and that it is too time consuming for a proper quality control. We think such objections are somewhat premature since both the conceptual soundness and the operational capability of the variety of biomonitoring systems have never been subjected to a thorough evaluation. From this it can be concluded that biomonitoring may have its drawbacks, but so has chemical monitoring. Therefore it seems reasonable to use biomonitoring when it does not overlap the physico-chemical data, thus indicating factors not testing for. If both types of testing are confirmatory, there is no justification to carry out both and the most cost-effective method should prevail, unless one is not disposed to invest in world-wide evaluation studies.

BIOMONITORING ACTIVITIES IN THE NETHERLANDS

Legislative framework

From Fig. 4 it can be derived that the geographical position of The Netherlands implies the necessity to combate water pollution on an international basis. The water quality of a large numer of Dutch waters is

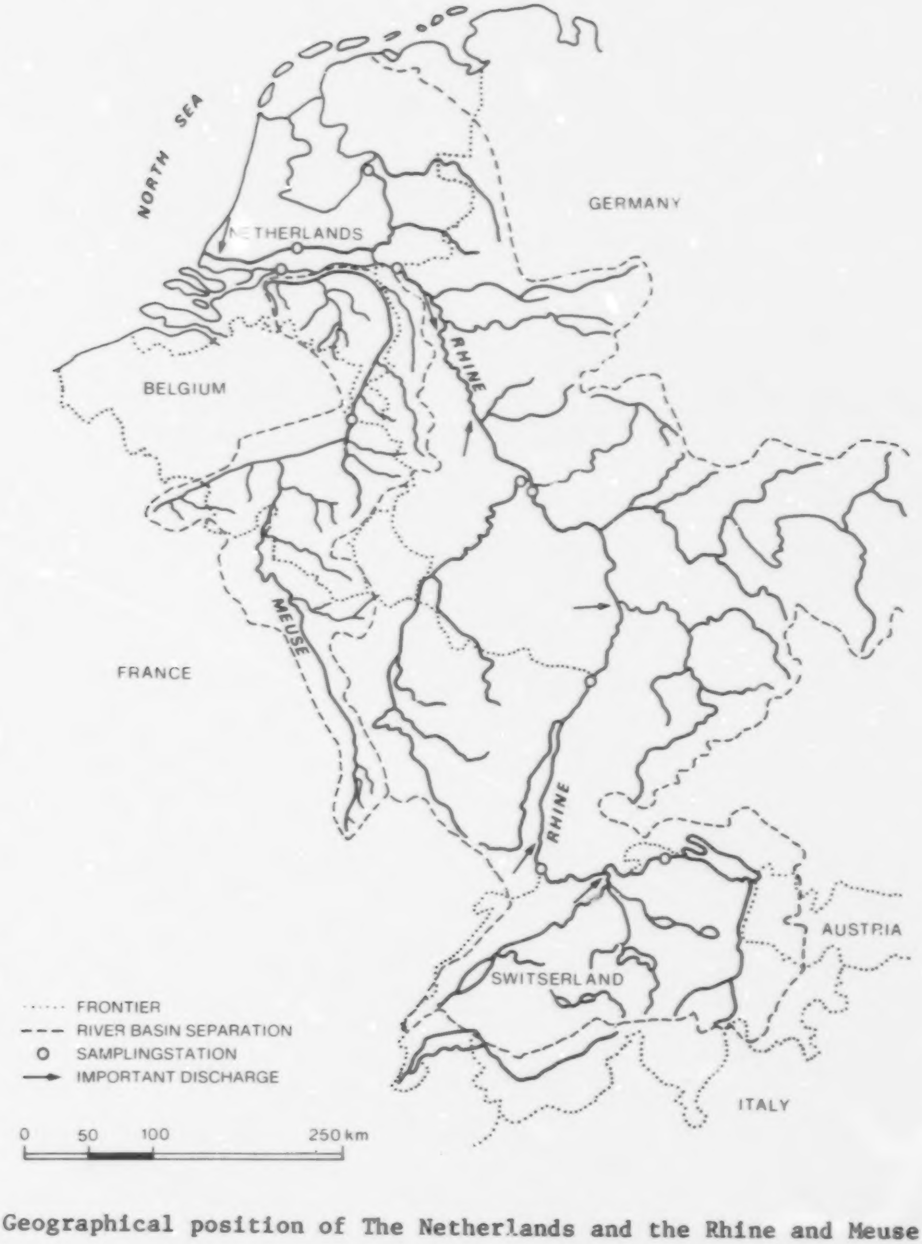
NORTH SEA
NETHERLANDS
GERMANY
BELGIUM
RHINE
MEUSE
FRANCE
RHINE
SWITSERLAND
AUSTRIA
ITALY
FRONTIER
RIVER BASIN SEPARATION
SAMPLINGSTATION
IMPORTANT DISCHARGE
0
50
100
250 km

Fig.4 Geographical position of The Netherlands and the Rhine and Meuse river basin.

affected to a considerable degree by the quality of the water of large rivers crossing the frontier. For example, about 65% of all the freshwater reaching the Netherlands is supplied by the heavily polluted river Rhine.

The national regulatory actions against waste disposal of the aquatic environment are based on several laws [1].

The confirmation with the directive 76/464/EEC and the convention against the chemical pollution of the river Rhine (1976) takes place in the framework of the Pollution of Surface Waters Act (1970). One of the principles on which the Act is based is decentralization. Central government is responsible for the management and quality of the major watercourses such as the rivers Rhine, Meuse and Scheldt and Lake IJssel and the Waddensea. The provincial authorities are essentially responsible for the remaining watercourses but they may in turn delegate responsibility to the water boards. This decentralization means that ways must be found of ensuring the necessary coordination and uniformity of planning, licensing and levy procedures. An important element in the coordination is formed by the "Water Action Programmes", which are revised every five years. The Act places at the disposal of the authorities responsible for pollution control a number of instruments by which their polices may be put into effect. Chief are the requirement of a permit for all discharges of polluting or harmful substances into surface water (for which biotesting may be required) and the possibility to prohibit or limit the discharge of certain substances. To meet the cost of pollution abatement a levy is imposed on those who discharge pollutants (oxygen-consuming substances, some heavy metals). Closely linked with the adoption of a number of international convention a number of amendments to the Act came intc fore in 1982, dealing with the following aspects:

- Discharges into municipal sewers, which may be made subject to licensing conditions where particular substances or establishment are involved.
- Limits will be placed on discharges of certain substances, i.e. on the maximum permissible quantity or concentration.
- A comprehensive system of water quality plans has been established in order to take account to the country's international commitments and to foster harmonized planning. Minimum quality objectives must be taken into account when the plans are drawn up and target dates for achieving them may be laid down nationally. The first plan is designed to implement the water quality objectives contained in the EEC directives relating to the abstraction of drinking water, water for bathing, fresh water for fish, the use of fish stocks and water for shellfish.
- The task of surveying current discharges and monitoring water quality is a substantial part of water quality control. The amendment to the Act lays down a number of rules on these points: surveys are to be carried out at least once every three years, primarily of blacklisted substances.

The provisions of the Oslo and London conventions on dumping at sea are implement at national level by the Pollution of the Sea Act (1977). In accordance with the conventions the Act contains a general prohibition on the dumping of several substances. In addition it is prohibited:

- To discharge polluting or harmful substances from a Dutch ship or aircraft anywhere in the world or to dump such substances from any ship or aircraft in Dutch territorial waters.
- To take substances on board in the Netherlands with the intention of dumping them.
- To deliver substances in The Netherlands with the intension of dumping them; unless an exemption has been granted by the Dutch authorities.

The Chemical Waste Act and Waste Disposal Act are designed to make and keep waste produced in (or imported into) the Netherlands subject to controls. Preference is accorded to reuse rather than destruction. Dumping or discharging at sea is favoured only if no practicable methods of processing on land are available and the environmental requirements are satisfied. As to the Chemical Waste Act a system of permits, exemptions and reporting has been set up by the central government to enable the flows to be controlled. In contrast to this, the implementation of the Waste Disposal Act is decentralized: it is the provinces that draw up plans for the disposal of designated waste materials. These plans must be approved by central government, however, which examines them in relation to the Act and the associated guidelines to ensure proper coordination of the various waste disposal plans.
Product standards may be laid down under both Acts (e.g. the Biodegradability of Surface-Active Substances in Detergents Decree under the Chemical Waste Act). These laws are thus designed to protect the environment directly or indirectly and may have an effect on the protection of fresh and salt water in ways not provided for by the Pollution of Surface Waters Act and the Pollution of the Sea Act.

Present activities

Biomonitoring of effluents in The Netherlands is restricted to discharges from pesticide formulating and manufacturing industry, mainly because of the batch-wise production process and the inadequate results of chemical testing on these rather complex wastes. Usually the biotesting is performed routineously at the basic level (Table II), but quantitatively fluctuating

Tabel II. A proposal for a sequential biomonitoring system and a choice of biological methods (see text).

Biomonitoring level	Effluents	Receiving waters
Basic level	indirect methods: short-term standardized tests or early warning systems depending on type of discharge	indirect methods: short-term tests (Microtox, Mutascreen) on fractions that are most (geno)toxic
Confirmatory level	indirect methods: extended battery of short-term tests and long-term tests, or direct methods: single-species studies*	direct methods: single species studies or community studies
Definite level	direct methods: community studies*	direct methods: extended community studies

* The direct methods on effluents have to be carried out in the receiving waters up- and downstream of the discharge point(s).

waste water is generally subjected to an extended battery of short-term and long-term tests (confirmatory level; Table II). In that case either the waste in it's whole or the "bad guys" are studied, depending on the complexity of the waste and knowledge about the chemical components. After these biological and chemical investigations, however, the effluent control is generally carried out chemically. The effluent biomonitoring is performed in the scope of licences by industry and the authorities, but on a very limited scale. As to receiving waters no legal biomonitoring activities take place, with the exception of those required by the EC-directive on dumping of titanium dioxide in sea. Yet, quite a number of biological monitoring studies are carried out by water management authorities and several research institutes in all types of surface waters and at different levels of biological organization. However, the activities suffer from a lack of defined water quality management directions. As a result the obtained data bases are often not directly comparable and sometimes even completely different.

Future activities

From the foregoing it can be concluded that a clearly defined national biomonitoring programme is lacking. In part this may be explained by the fact that there was no clear perspective on biomonitoring at the time the environmental laws were prepared. From the impressive amount of papers dealing with biomonitoring it can be derived that a wide variety of biological methods have been applied to serve the purposes outlined before. In most papers, however, the method and results are given without clarifying the objective. As a result, there was no consensus on what a biological indicator is really indicative for [10].

It is only recently that there has been some integration of papers dealing with aspects such as principles, objectives, methods and strategies [5, 34, 51] and several research needs have been identified. However, Macek [29] correctly stated that the legislative framework is repressive to research efforts. Regulators tend routinely to ask for the same kinds of data and cannot assimilate other types of data into the decision process. From their point of view there is only a quantative problem, and so the scientific community in the field has hardly any room to conduct significant research outside this framework. Still such research efforts are necessary to give the scientific back-up to influence the regulatory policy. The present OECD activities may help to break through this vicious circle by stimulating and coordinating concerted international efforts in determining the value of biomonitoring.

A PROPOSAL FOR A STEP-WISE PROCEDURE

Need for setting priorities

In case of toxic substances, biomonitoring should be based on the measurement of (a) deleterious effects specific for toxity, (b) which are of ecological significance, (c) can be measured accurately, (d) with a high signal to noise ratio, (e) with a high response rate, (f) which are perceptible over a wide range of stress intensity, (g) which are reversible, (h) can be expressed in terms comprehensible to decision-makers and (i) can be assessed at relatively moderate costs [51]. In practice, some of these criteria are not consistent with each other since their fulfillment is directed by different levels of biological integration. Hence, there is no such thing as an universal biomonitoring system.

Every approach will be a compromise and depends on the information most needed. In Table I the advantages and limitations of two different biomonitoring approaches are summarized. The first approach is based on direct observations on the state of health of the biota naturally occuring in the environment to be judged; the second approach is based on indirect observations, determining the water quality by examinations of the state of health of organisms experimentally exposed to the water under controlled conditions. Since both methods are complementary, it is recommended to apply them simultaneously to obtain information needed for a proper water quality management, that is e.g. information on the ecological state of surface waters in relation to possible causative factors such as toxic effluents [44]. It is in this respect that it is felt that application of biological monitoring would be enhanced if we succeed in establishing an integrated ecotoxicological approach in a manner similar to the step-wise predictive hazard evaluation system for chemicals.

For biomonitoring, however, such a construction will be more complex and will meet more technical difficulties since several objectives have to be fulfilled and different parties will be involved. Therefore it is a necessity to set priorities in evaluating the application possibilities of biomonitoring in decision making processes. The most logical to do this is from a regulatory point of view. This reduces the efforts to monitor point-source discharges and receiving waters. Concerning the other application possibilities, it is felt that it is industry's own responsibility to make business investments in biomonitoring that could be cost-saving in the long run. As far as water users like public water supplies concerns, the application of additional biomonitoring techniques to detect accidental spills is considered questionable in most cases. Environmental toxicant concentrations are even than below the detection limit of

Tabel I. Advantages (+) and disadvantages (-) of the direct (field studies) and indirect (laboratory studies) method for determining water quality related to toxic pollution [44].

Research method		Ecological relevance	Signal to noise ratio	Specificity	Response rate	Response range	Indexability	Cost
Direct	community studies	+ +	- -	- -	- -	+ +	+	-
	single species studies	+	±	±	±	±	- -	-
Indirect	long term single species studies	±	+	±	±	±	- -	-
	short term single species studies	- -	+ +	+ +	+ +	- -	+ +	+

biomonitoring systems, whereas calamities will often be discovered by fish kills upstream of the monitor location before harmful conditions are detected by the biomonitors [41].

A basic, confirmatory and definite level

The basic principle learned from the ecotoxicological hazard assessment, that each increase in sensitivity or ecological representativeness will automatically result in increased costs [37], is also applicable to biomonitoring. Therefore we suggest to recommend to follow a similar step-wise procedure including a basic, a confirmatory and a definite level. In Table II a proposal for a general framework for applying biomonitoring at different levels is given. In our view the tests selected for the basic level should be indirect methods of the highest simplicity (see Table I). Depending on the biomonitoring objects, the confirmatory level could comprise direct (receiving systems) or indirect (effluents) methods of a more complicated nature. The definite level should give information that is of ecological relevance, indicating the application of direct methods only.

In developing guidelines, however, it is important to realize that monitoring activities should not represent a static process, but must be dynamic and flexible in practice, addressed directly to the situation or water body being examined. For example, the type and frequency of effluent biomonitoring will depend on the type of effluent in terms of quantative and qualitative aspects and the variation therein in time. Biomonitoring of receiving water bodies may differ widely as to their functions and their geomorphological, hydrological, physico-chemical and biological characteristics, whereas the spatial distribution of pollutants sources and water flow will largely determine the number and locations of sample sites, sample intervals and so on. As a consequence a base-line study will be indispensible to determine the significance of the biomonitoring activities at the various levels, e.g. with respect to bioaccumulation and biodegradation. Hence, it is not likely that there is a standard biomonitoring method optimal for all situations to serve the same purpose, especially at the confirmatory and definite levels of monitoring applying direct methods. Therefore, the present efforts should be aimed at the establishment of a more organized and systematic, and not necessarily a uniform, approach to biomonitoring. In this respect much relevant information could and should be obtained form the numerous papers on the design of regulatory water quality monitoring programs [3, 5, 8, 9, 14, 17, 20, 27, 33, 39, 40, 52]. Although most of these papers are traditionally aimed at chemical monitoring, the approach is essentially the same as they deal with aspects as network design, sample collection, laboratory analysis, information utilization and decision making. The monitoring system matrix as suggested by Ward [52], for example, may provide a basis upon which a thorough approach to managing, evaluating and eventually optimizing biomonitoring can be developed.

There are many uncertainties that have to be cleared and questions to be answered before biomonitoring is feasible in water pollution control, and most of these are not of scientific nature. In fact, given the current financial squeeze, there is a real danger that the quality and reliability of present monitoring will even deteriorate in years to come [35]. Even if resources were available, legislation to enable the authorities to require biological research is exceptional, whereas it is known that water quality biomonitoring programs will usually only be carried out to fill legal obligations [36] and industry will not likely use biomonitoring unless required to do so by law [5].

Further there are management problems to overcome. For example, who will generate, analyze, collect, administrate and use the biological data? How are these data acquisition and data utilization designed in order to safeguard the use of accurate, representative and comprehensible data in an efficient and effective way? How to harmonize these activities in such a way that the information output is exchangable amongst different parties in a (bio)monitoring network without breaking into matters like confidentiality? These aspects deserve at least equal attention as the scientific soundness of biomonitoring.

Definitely, the use of a step-wise biomonitoring scheme implies the necessity of a scoring procedure in order to decide at which level biomonitoring should take place. For example, for industrial effluents this may hold a formula incorporating factors like discharge volumes, toxity of discharge, presence of bioaccumulative substances and the actual dilution factor. As to municipal effluents, the inhabitant equivalent could also play a role. As far as the biomonitoring of receiving waters is concerned, the levels could be linked with factors like the functional objectives of these waters, the catchment area and estimates on pollution loads from both industrial and domestic point sources as well as from diffuse sources such as agricultural run-off of pesticides and fertilizers. However, with regard to the query on the possibility of fitting biomonitoring into the regulatory framework, a discussion about this is still considered as premature.

COMPLETEMENT OF THE BASIC LEVEL

Taking into account the complexity of biomonitoring, it is logical to focus the attention on the completion of the proposed basic level . Not only as it is a first step, but also since it is most likely that scientists reach a concensus on the type of biomonitoring at this level within reasonable time, whereas the costs and limits of expertise will not likely prevent decision makers from implementing biomonitoring.

Biomonitoring of effluents

As stated before, the objective of effluent monitoring is to protect receiving systems from harm as much as possible and, biomonitoring is only needed if it provides information not tested for by chemical monitoring or if it is more cost-effective in providing the information. Hence, biomonitoring should be applied in case the effluents are of complex nature, vary quantitatively or qualitatively, or contain pollutants that can not be measured chemically because appropriate methodology does not exist, or are present in concentrations below the chemical detection capability. However, since in most cases case-studies are still necessary to proof the superiority of either chemical or biological methods as to reliability and cost-effectiveness, a concensus should be reached on a biomonitoring strategy for the different types of effluents, as there are effluents of more or less constant chemical composition, effluents fluctuating only quantitatively in chemical composition and effluents fluctuating both qualitatively and quantitatively. As to the first two types, the basic level could include the short-term toxicity testing according to the OECD guidelines once and subsequently the use of an automatic early warning system to prevent accidental spills. As to the third type of effluent, only the use of battery of a continuous automatic biosensors makes sense.

In contrast to this, such a device will be of no use in case of batch-wise effluents; in those situations the basic level may hold a toxicity screening of the batch using acute bioassays with algae, crustaceans and fish. Based on the information obtained on the toxicity of the batch it can be decided whether or not the batch can be discharged as such or not. Definitively, such a decision is not possible without additional technical modifications such as storage basins.

As far as the test methods concerns, the acute single-species tests need no further discussion since they are well defined in the present OECD guidelines. However, these guidelines have been developed primarily in order to assess the environmental impact of individual chemicals. When the same test procedures are used to assess the hazardousness of waste water discharges, a number of difficulties may be encountered:

(a) In most cases the "chemical content" of the waste is not known. Biodegradation studies may therefore be analyzed by sum-parameters like COD, BOD, EOCl etc. These analyses may be indicative for the bulk of the chemicals but don't allow statements about the individual components, nor about compounds not included in the sum-parameters.

(b) Whole waste accumulation testing is hardly applicable, since it is not known which substances should be looked for. Such testing may lead to the conclusion that the waste as a whole is hardly bioaccumulative, whereas compounds could be present with a strong tendency for accumulation. If signs for bioaccumulation are derived e.g. by plotting LC_{50} values in time, long-term toxicity testing should be considered.

(c) Toxicity testing of waste water as such may be difficult for a number of causes:

- extreme pH-values in comparison with pH-values acceptable for the test organisms. Neutralization of the waste may result in high salt contents and co-precipitation of bioactive substances,

- the presence of suspended solids may exert adverse effects on test species such as filter-feeders (Daphnia),
- excessive salinity or unsuitable hardness may affect the test organisms,
- if volatile substances are present, the test solution can't be aerated without loss,
- if strongly adsorbing substances are present, precautions should be taken to minimize adsorption losses,
- if readily biodegradable substances are present, oxygen may be depleted during the toxicity testing.

Although each of these difficulties may be overcome, they will impose problems in the interpretation of the data. As to automatic and continuous biomonitoring devices, these need a proper evaluation regarding the conceptual soundness before these methods will be accepted by industry and regulatory agencies. The review paper of Cairns and Van der Schalie [7] may provide the basis upon which future attempts can be made for further evaluation.

Biomonitoring of receiving waters

As proposed in Table II, the basic level of biomonitoring receiving water bodies requires simple and shortlasting indirect methods, with the objective to obtain information on the water quality in terms of (geno)toxicity in both space and time. However, taking into account the pollution levels in most surface waters, acute effects will (fortunately) not occur and laboratory experiments on surface water will be too time-consuming and too expensive to be performed on a routine base. A recently developed feature is to apply a technique to concentrate (geno)toxic compounds from the water to speed up the response rate. This also enables the testing of several concentrations in order to determine a concentration-response relationship. In The Netherlands this method has been succesfully applied to assess the degree of hazardous chemical pollution of surface waters in terms of toxicity and mutagenicity [25, 47]. These socalled short-cut methods may be criticized on two points: the choise of test species and that of concentration/isolation technique. The Ames test is generally considered as the most practical technique to screen for genotoxicity and will probably not be a point of discussion. To screen for aquatic toxity, however, there is presently no concensus on the choice of test species based on observations that the sensitivity to toxicants is highly variable amongst different species, even more than a factor 20,000 [13, 21, 22, 43, 45, 50]. Yet, it should be realized that these differences in species susceptibilities are much smaller when the relative susceptibility of the species based on weighing the toxicity data on a number of chemical substances are taken into account (Fig.5). This indicates that in case of surface waters polluted with a variety of chemical pollutants the difference in response level between different species is likely to be small. This hypothesis is supported by observations on the acute toxic effect of Rhine water concentrates on a number of representatives of "keystone" groups for the whole range in the saprobian system as showed in Table III [42]. Therefore, it is recommended to use the most practicable and standardized methods. In this respect testing systems like the Microtox (Beckmann°; a luminescent bacterial test system [11, 30, 38]) and the recently developed Mutascreen (Labsystems° [1]) may very well suit the purpose since they yield fairly reproducible results comparable to those derived from usual test systems, do not require culturing of test organisms, are standardized and easy to handle, whereas the performance takes far less time than the usual test systems. Therefore it is recommended to encourage the further development of such systems.

A drawback of applying a concentration technique is that all concentration

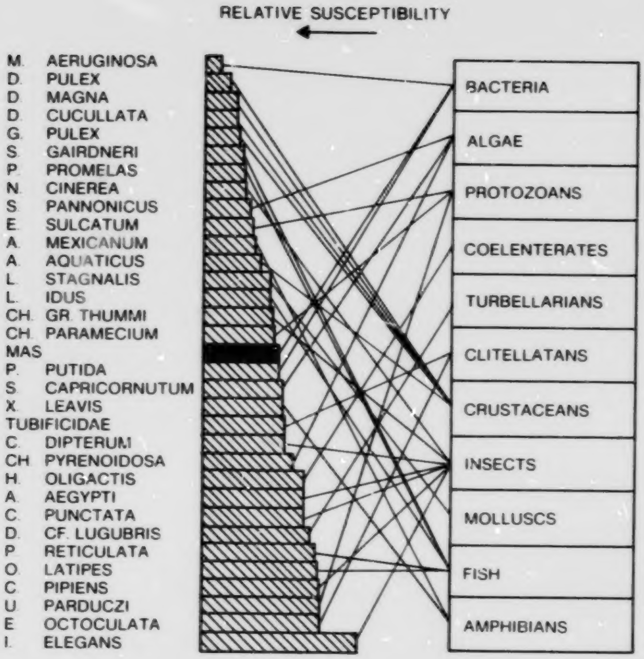

Fig.5 The relative susceptibility of various species to toxicants based on acute toxicity levels for 15 different chemical substances. Hereto the average no observed effect level was determined for each compound and subsequently for all species the geometric mean of these ratios was compared with that of the most susceptible species. Note the sensitivity of the Microtox Assay System (black bar) (after [42, 45, 53]).

Table III. The susceptibility of benthic invertebrates, representative for the whole range in the saprobian system, to a mixture of organic compounds concentrated from water of the river Rhine [42]. The species are ordered according to their supposed tendency to disappear as degree of organic pollution increases.

Species		Concentration factor which results in 50% mortality in 48 h (LCF50)
Oligochaeta	Tubificidae	159
Diptera	Chironomus gr. thummi	144
Isopoda	Asellus aquaticus	158
Amphipoda	Gammarus pulex	192
Trichoptera	Athripsodus cinereus	124
Ephemeroptera	Cloëon dipterum	184
Plecoptera	Nemoura cinera	214

procedures are selective for one or several physico-chemical properties and thus will alter the chemical composition of the water. Since application of an array of complementary concentration methods may not be economically feasible, attention could be given to the method that proved to be the most effective in concentrating the hazardous fraction.

CONCLUSIONS AND RECOMMENDATIONS

- It is recommended to use a comprehensive definition of biomonitoring: the use of biological responses to evaluate (changes in) the hazardousness of effluents and receiving waters with the intent to use this information in a quality control program. This excludes the measurement of pollutant concentrations in biological materials (chemical monitoring).
- In The Netherlands there is only a limited base on which biomonitoring activities are performed within the regulatory framework. Many biomonitoring studies are performed outside this framework, but a clearly defined national plan is (still) lacking.
- It is recommended to use biomonitoring as a tool to signal, predict and control water pollution in those cases biomonitoring is more cost-effective than physico-chemical monitoring, or in case it indicates factors not testing for.
- From a practical point of view a step-wise procedure is recommended, including a basic, confirmatory and a definite level. Taking into account the complexity of biomonitoring, at first attention should be given only to the completion of the basic level and the application possibilities of short-term toxicity methods therein.

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Biological Testing of Effluent

- current state in Japan and a proposal of relevant test procedure-

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Present-day life entirely supported by modern science and technology gave rise to new type of effluents and introduction of many man-made chemicals into environment brought about unknown dangers and risks. Actually some of these have been revealed to present an adverse effects on human health and environment. Especially the aquatic environment as a final destination of effluents are receiving enomous amount of pollutants produced by man's activities - industrial, agricultural and domestic etc.. Their deleterious effects on the aquatic ecosystem have been very much concerned to cause destruction of natural resources. They may finally reach to human being through food web in ecosystem. Most foods obtained or harvested from waters, marine or inland, are subject in varying degrees of environmental pollution. Some of the pollutants, even in slight or low ambient level, can be built up in the biota and tend to concentrate in the higher life forms including man.

Ecological imbalance can also be resulted when some organisms , such as certain single cellular algae undergo population explosion and form huge biomasses. Which can directly cause killing of aquatic organisms dwelt and also be attributed to secondary pollution.

In order to prevent such risks in human being and environment, the Basic Law for Environmental Pollution Control was enacted in Japan in 1967 for water pollution and 6 other typical environmental pollution such as air pollution, noise, vibration, ground subsidence, offensive odour, and soil contamination. The responsibilities of national government, regional government, and industries were clearly illustrated in the Basic Law and the Environmental Quality Standards were established under the Article 9 of the Basic Law :

> "With regard to environmental conditions relating to air, water,and soil pollution, and noise, the Government shall establish environmental quality standards, the maintenance of which is desirable for the protection of human health and the conservation of the living environment".
>
> "The Government shall make efforts to ensure the maintenance of the above-mentioned standards, by implementing environmental pollution control measures in a comprehensive, effective and appropriate manner".

The Environmental Quality Standard for water pollution covers following nine toxic substances and nine items now; cadmium, cyanide, organic phosphorus, lead, hexavalent chromium, arsenic, total mercury, alkyl mercury, PCBs, and hydorogen ion concentration (pH), biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), dissolved oxygen (DO), number of coliform groups, n-Hexane extracts, total phosphorous, total nitrogen. (Table 1, 2-1, 2-2-1, 2-2-2, 2-3)

Table 1 Water quality standards related to the protection of human health:

Item	Standard Values*
Cadmium	0.01 ppm or less
Cyanide	Not detectable
Organic phosphorus**	Not detectable
Lead	0.1 ppm or less
Chromium (VI)	0.05 ppm or less
Arsenic	0.05 ppm or less
Total mercury	0.0005 ppm or less
Alkyl mercury	Not detectable
PCBs	Not detactable

Notes * Maximum values. But with regard to total mercury, standard value is based on the yearly average value. (1974 –)

** Organic phosphorus includes parathion, methyl parathion, methyl dimeton and E.P.N.

Note: JIS K0102: Japan Industrial Standard for testing and measurement of waste water.

Table 2 Water quality standards related to the conservation of the living environment.

Table 2-1 Rivers

Category	Item / Purpose of water use	Standard Values*				
		pH	Biochemical Oxygen Demand (BOD)	Suspended Solids (SS)	Dissolved Oxygen (DO)	Number of Coliform Groups
AA	Water supply, class 1; conservation of natural environment, and uses listed in A-E	6.5-8.5	1 ppm or less	25 ppm or less	7.5 ppm or more	50 MPN/100 mℓ or less
A	Water supply, class 2; fishery, class 1; bathing; and uses listed in B-E	6.5-8.5	2 ppm or less	25 ppm or less	7.5 ppm or more	1,000 MPN/100 mℓ or less
B	Water supply, class 3; fishery, class 2; and uses listed in C-E	6.5-8.5	3 ppm or less	25 ppm or less	5 ppm or more	5,000 MPN/100 mℓ or less
C	Fishery, class 3; industrial water class 1; and uses listed in D-E	6.5-8.5	5 ppm or less	50 ppm or less	5 ppm or more	
D	Industrial water, class 2; agricultural water**; and uses listed in E	6.0-8.5	8 ppm or less	100 ppm or less	2 ppm or more	
E	Industrial water, class 3; conservation of the environment	6.0-8.5	10 ppm or less	Floating matter such as garbage should not be observed.	2 ppm or more	

* The standard value is based on daily averages. (The same applies to the standard values for lakes and coastal waters.)

** At the inlet of irrigation water, pH shall be between 6.0 and 7.5 and dissolved oxygen shall not be less than 5 ppm.

(The same applies to the standard values for lakes.)

Table 2-2-1 Lakes (natural lakes, reservoirs, marshes and man-made lakes with more than 10 million cubic meters of water)

Category	Item / Purpose of Water Use	Standard Values				
		pH	Chemical Oxygen Demand (COD)	Suspended* Solids (SS)	Dissolved Oxygen (DO)	Number of Coliform Groups
AA	Water supply, class 1; fishery, class 1; conservation of natural environment, and uses listed in A-C	6.5 - 8.5	1 ppm or less	1 ppm or less	7.5 ppm or more	50 MPN/100 mℓ or less
A	Water supply, class 2 and 3; fishery, class 2; bathing, and uses listed in B-C	6.5 - 8.5	3 ppm or less	5 ppm or less	7.5 ppm or more	1,000 MPN/100 mℓ or less
B	Fishery, class 3; industrial water, class 1, agricultural water, and uses listed in C	6.5 - 8.5	5 ppm or less	15 ppm or less	5 ppm or more	-
C	Industrial water, class 2; conservation of the environment	6.0 - 8.5	8 ppm or less	Floating matter such as garbage should not be observed.	2 ppm or more	-

* With regard to fishery, classes 1, 2 and 3, the standard value for suspended solids shall not be applied for the time being.

Note: See notes for Rivers.

Table 2-2-2 Environmental Water Quality Standards on Nitrogen and Phosphorus in Lakes and Reservoirs

Item / Category	Purpose of utilization	Standard values	
		Total Nitrogen	Total Phosphorus
I	Conservation of natural environment, and uses listed in II-V	0.1 mg/l or less	0.005 mg/l or less
II	Water supply classes 1,2 and 3 (excluding special types); Fishery type 1, bathing and uses listed in III-V	0.2 mg/l or less	0.01 mg/l or less
III	Water supply class 3 (special types), and uses listed in IV-V	0.4 mg/l or less	0.03 mg/l or less
IV	Fishery type 2, and uses listed in V	0.6 mg/l or less	0.05 mg/l or less
V	Fishery type 3; industrial water; agricultural water; conservation of the living environment	1 mg/l or less	0.1 mg/l or less

Remarks:
1. The standards are measured in terms of annual averages.
2. The standards for total phosphorus are not applicable to agricultural water uses.

Notes:
1. Conservation of natural environment - Conservation of scenic points and other natural resources.
2. Water supply class 1 - Water treatment by simple cleaning operation such as filtration.
3. Water supply class 2 - Water treatment by normal cleaning operation such as sedimentation and filtration.
4. Water supply class 3 - Water treatment by sophisticated cleaning operation including pretreatment. ("special types" mean water treatments by special cleaning operation in which removal of smelling substances is possible.)
5. Fishery type 1 - For aquatic lives such as fish of the salmon group and sweet fish, and for those of fishery type 2 and 3.
6. Fishery type 2 - For aquatic lives such as smelt and for those of fishery type 3.
7. Fishery type 3 - For aquatic lives such as carp and silver carp.
8. Conservation of environment - Up to the limits at which no unpleasantness is caused to the people in their daily lives(including a walk along the shore).

Table 2-3 Coastal Waters

Category	Item / Purpose of Water Use	Standard Values				
		pH	Chemical Oxygen Demand (COD)	Suspended Solids (SS)	Number of Coliform Groups*	N-hexane Extracts
A	Fishery, class 1; bathing; conservation of natural environment, and uses listed in B-C	7.8 - 8.3	2 ppm or less	7.5 ppm or more	1,000 MPN/100 mℓ or less	Not detectable
B	Fishery, class 2; industrial water, and uses listed in C	7.8 - 8.3	3 ppm or less	5 ppm or more	-	Not detectable
C	Conservation of the environment	7.0 - 8.3	8 ppm or less	2 ppm or more	-	-

* With regard to the quality of fishery, class 1 for planting oysters, the number of coliform groups shall be less than 70 MPN/100 mℓ.

Notes: 1. Fishery, class 1: For aquatic life such as red sea-bream, yellow tail, seaweed and those of fishery, class 2.
Fishery, class 2: For aquatic life such as gray mullet, laver, etc.

2. Conservation of the environment: Up to the limits at which no unpleasantness is caused to people in their daily life (including a walk by the shore, etc.)

Industrial and other sources effluents are controled by the Water Pollution Control Law and the effluent standards to meet the environmental standards are applied to the sources designated by the law. (Table 3-1, 3-2)

Moreover, a total discharge control in the closed or semiclosed sea has been introduced to improve the water quality by the total discharged amount of COD instead of its concentration. The legislative action had already been undertaken in the Tokyo Bay, the Osaka Bay, the Ise Bay, and the Seto Inland Sea.

Thus, by the facts mentioned above, the water quality of the public water has been gradually improving throughout Japan. Especially, the human health items have almost completely met the Environmental Quality Standards now.

As the Environmental Quality Standards are limited, there might be, however, ineffectiveness to the substance which interfere with cellular and subcellular process in the organism at far below detection limits in chemical analysis to such an extent that disrupt physiological or behavioural activities but do not cause immediate mortality, although death may follow because of interference with feeding and reproductive activities or other abnormal behaviour, or with movements to obtain food or escape from predators.

In Japan, no biological testing for effluent discharge has been employed for a regulatory purpose. But, quite a few researches have been continued about biological testing.

Hence, we would like to propose some procedures to be able to meet such difficulties mentioned above in a different manner

Table 3 Effluent standards

Table 3-1 Substances related to the Protection of Human Health

Toxic Substances	Permissible Limits	Method of measurement
Cadmium and its compounds	0.1 mg/1	JIS K0102 40.2
Cyanide compounds	1 mg/1	JIS K0102, 29.1.2.1.3
Organic phosphorus compounds (parathion, methyl parathion, methyl demeton and EPN only)	1 mg/1	JIS K0102, 23
Lead and its compounds	1 mg/1	JIS K0102, 39.2
Sexivalent chromium compounds	0.5 mg/1	JIS K0102, 51.2.1
Arsenic and its compounds	0.5 mg/1	JIS K0102, 48.1 or 48.2
Total mercury	0.005 mg/1	*
Alkyl mercury compounds	Not detectable	*
PCBs	0.003 mg/1	*

JIS : JAPAN INDUSTRIAL STANDARD
K0102: Testing method for industrial waste water

* Set by notification of Environment Agency, No. 59

Table 3-2 Items related to the Protection of the Living Environment

Items	Permissible Limits	Method of measurement
pH	5.3 to 3.6 for effluent discharged into public water bodies other than coastal waters/5.0 to 9.0 for effluent discharged into coastal waters	JIS K0102, 8
BOD, COD	160 mg/l (daily average 120 mg/l)	JIS K0102, 16 JIS K0102, 13
SS	200 mg/l (daily average 150 mg/l)	
N-hexane extracts	5 mg/l (mineral oil) 30 mg/l (animal and vegetable fats)	
Phenols	5 mg/l	JIS K0102, 20.1, 20.2
Copper	3 mg/l	JIS K0102, 37.2
Zinc	5 mg/l	JIS K0102, 38.2
Dissolved iron	10 mg/l	JIS M0202, 3.1.4.(2) and JIS K0102, 47.2
Dissolved manganese	10 mg/l	JIS M0202, 3.1.4(2) and JIS K0102, 46.2
Chrome	2 mg/l	JIS K0102, 51.1.1
Fluorine	15 mg/l	JIS K0102, 28
Number of coliform groups (per cc)	3,000 (daily average)	

Note: °The discharge standards in this table are applied to the effluents from industrial plants and other places of business whose volume of effluents per day is not less than 50 m^3.

°The discharge standards for BOD are applied to public waters other than coastal waters and lakes, while the discharge standards for COD are applied only to effluents discharged into coastal water and lakes.

from the Mussel Watch in U.S. EPA.

I. Biological monitoring of effluent

Control or monitoring of effluent discharged are depended largely upon chemical or physical analysis. Such measurements have a detection limit. Furthermore, such measurements cannot predict the toxic effects consorted with multiple components of the effluent which may act synergistically or antagonistically with one another. A biological monitoring system, functioning in continuously, is not only able to integrate these effects, but also to provide an advance warning of potential risks.

An improved biological monitoring system based upon fish activity responses to intoxication has been developed by Morgan et. al. (1982) using ultrasonic technics. The monitoring equipment was operated automatically on-line to detect potentially harmful substances in flowing water. The system monitors the activity of the fish by means of ultrasonic echoes, which in a chamber equiped with ultrasonic emitter and receiver. Fish movement can be monitored by light beam interruption, which had been employed by Waller and Cairns (1972), however, it cannot be used for turbid water like industrial or domestic effluents. It is the great advantage to use the ultrasonic sensor compared with light beam one. The fish utilized for biological monitoring system is to be the most sensitive one to intoxication by the effluent among indigenous species in the stream. The critical concentration of the effluent is also to be examined prior to use. The total cost of installing the biological monitoring system in plant was approximately U.S.$27,500. The cost of hardware for the electronic monitoring and alarm units was approximately U.S.$6,200. Running expenses for one year operation and maintenance was only U.S.$2,800. which may be comparably favourable with the the chemical analysis of the effluent.

The system was applied to monitoring of effluent in the pulp and paper mill plant situated in the Tugela River, South Africa. The results obtained during twelve month period were reported to be satisfactory to the detection and control of acutely toxic materials which would have deleterious effects upon the receiving ecosystem. There are, however, necessities for assessing the adequacy of parameters used in this system in different cases and also for further improvements in its data acquisition and evaluation devices.

There is one example where biological monitoring has been employed in a non-regulatory level, though it is a form of recommendation for newly constructed factory, in Yokohama City, Japan. It is an extensive manner in biological monitoring as to provide a pond in the stream between the waste water treatment plant and its discharging point , in which fish are kept and subjected to observation. It is aimed to get early warning of discharges of toxic substances, and protect the aquatic environment. The pictures show the biological monitoring pond where kept a shoal of colored carps in continuous exposure to the effluent from the film manufacturing factory in Kanagawa Prefecture, Japan. (Fig. 1).

Installation of continuous monitoring in this factory, Fuji Film Company, has been accomplished in 1973. Since then , accidental discharge of toxic substances never heard in this factry for 11 years. The cost of 6 series hardware for electric monitoring (TV camera, CRT, cable and cage etc.) is estimated approximately U.S. $ 208,000 now. Running expenses for one year operation and maintenance are U.S. $ 16,700, including expenses for person and fish feeding.

These are considered as a kind of security for preventing the accident. The benefits coming to industry, from the installation of the system, are (1) advancing of environmental concern for member (effective education of member) and (2) favourable propaganda to visiters (10,000 cap./y), but it is more difficult to assass.

Running cost is considered small compare with propaganda cost and various benefits in this company. These favourable effects from monitoring system are resulted from big scale one.

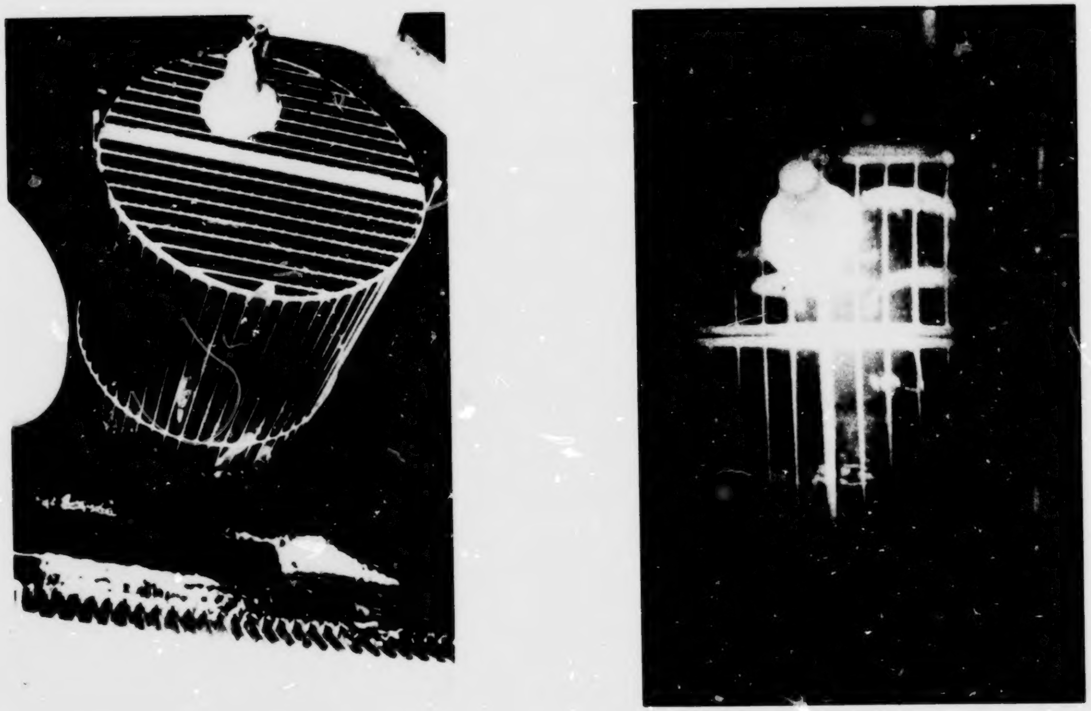


Fig. 1 Monitoring fish cage and TV camera installed in drainage channel and CRT observation.

II. Proposal of an algal test observing the photosynthetic activity of Porphyra sp. using radio isotope technics

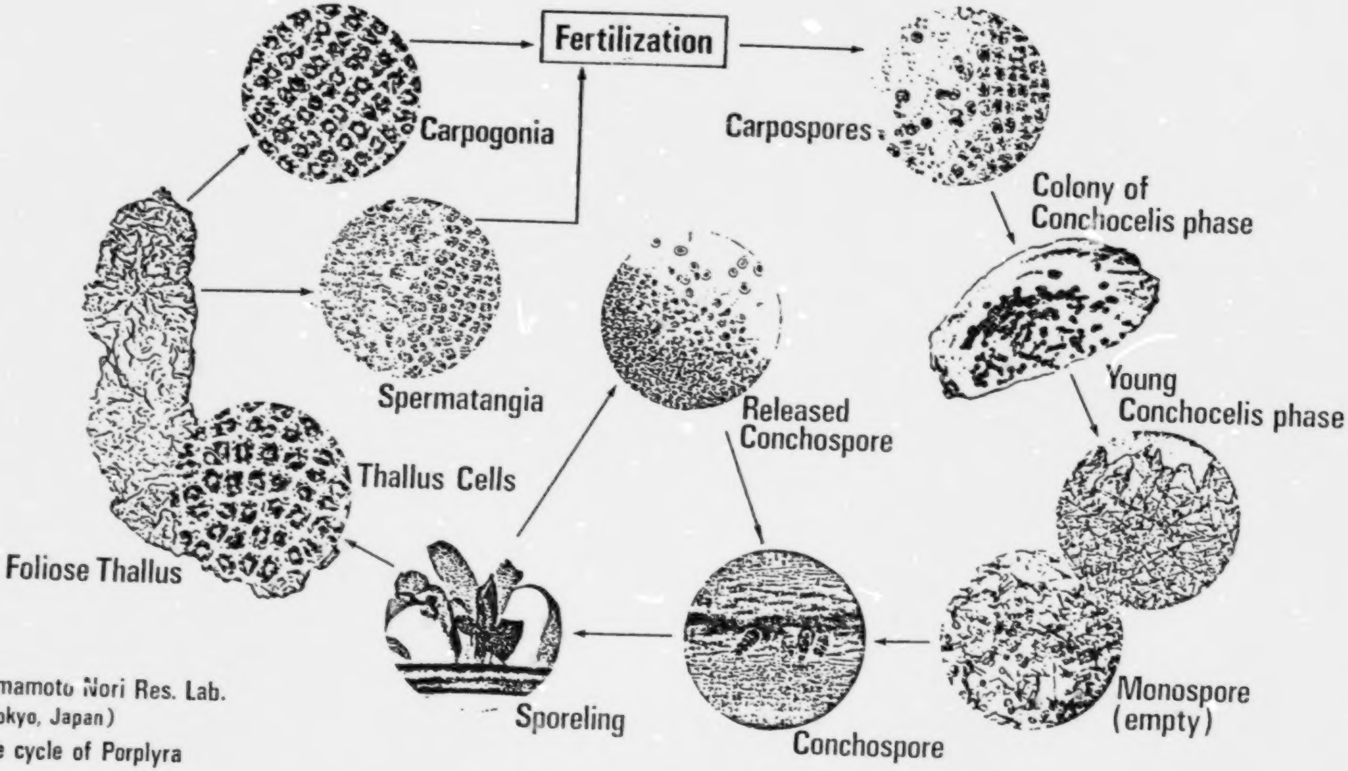
For evaluation of hazards of effluent on ecosystem, a relevant biological testing procedure should be provided with special consideration on its complexity and uncertainty mentioned before. A considerable number of biological tests have been developed with both individual and multiple species to assess the toxicity of chemicals. Most of them, however, were not based on the ecotoxicological concept. The Ecotoxicology Group of OECD Chemical Testing Program proposed several toxicity tests for assessing the effects of chemicals on ecosystem. Three basic tests among them were included in the Minimum Premarketing Set of Data (MPD), i.e. algal growth inhibition test, daphnia acute and reproduction test, and fish acute toxicity test. Preliminary assessment of hazards to the ecosystem on its structure and function could be made by these tests. More elaborate test, however, should be necessary on a wide range of species in order to evaluate the risk of ecological damage. In this respect, aquatic macrophytes are of important candidate, because they share primary production with phytoplankton in aquatic environment. A laver,Porphyra sp., is commonly distributed in the coastal water of the Temperate Zone, and is the most important species in marine aquaculture in Japan.

Toxicity test using Porphyra sp. has been conducted by observing its growth rate, cell conditions, photosynthetic activity, and its certain constituents. Among them, photosynthetic activity may be accurately reflected the effects of chemicals exposed. This measurement is made by analyzing either the rate of oxygen production or assimilation of carbon dioxide by the plant.

A study introduced here was carried out aiming to develop a relevant biological test procedure for evaluation of the effects of chemicals on an algal photosynthetic activity using radio isotope technics. It can be applied to the effluent as well. In order to minimize the deviation of observed results and to secure their reproducibility, physiologically homogenous specimen were provided by acclimatization of algae under controlled test conditions, and by synchronizing the exposure to test chemicals with their biorhythm. Here some results applied the procedure to some chemicals and effluents of sewage treatment plant are demonstrated.

The organisms tested, laver (Porphyra yezoensis) were supplied by Laboratory of Phycology, Tokyo University of Fisheries where they are cultured continuously throughout the life cycle of the algae. (Fig. 2) The strain was preserved in refrigerator in the conchocelis stage, a kind of resting spore in an oyster shell. Before the test, germination process have to be conducted by raising the water temperature to 13 °C in an artificial seawater medium. The germs are cultured in the medium at 13 °C under the illumination of a white fluorescent lamp (4,000 lux, 10 hours of light and 14 hours of darkness in a day) for two weeks, with aeration (200 ml/min.). At this stage algae of about 1 cm leaf length were further acclimataized for 1 week with 10,000 lux illumination. Final leaf length was approximately 3 cm.

Fig. 2 LIFE HISTORY OF NORI


Fertilization
Carpogonia
Carpospores
Colony of
Conchocelis phase
Spermatangia
Released
Conchospore
Young
Conchocelis phase
Thallus Cells
Foliose Thallus
Sporeling
Conchospore
Monospore
(empty)

Yamamoto Nori Res. Lab.
(Tokyo, Japan)
Life cycle of Porplyra

Substances tested were pentachlorophenol(PCP),3-(3,4-dichlorophenyl)-1,1,-dimethylurea)(DCMU), Sodium linear dodecilbenzen sulfonate(C12-LAS), and an effluent obtained from the Sibaura Sewage Treatment Plant. Purity of the chemicals used were 99.0%.

Three sample lavers were exposed to the chemicals or effluent in a Erlenmeyer's flask with 100 ml or 200 ml of culture medium. Concentration or dilution ratio of substances to exposure was decided from the results of preliminary experiment or literatures. A 100 μl of $Na_2{}^{14}CO_3$ (specific activity 10 μCi/ml) was added simultaneously with substance to be tested into the test flask after two hours illumination which was a kind of stabilization period for photosynthesis observed previously. The ^{14}C activity both assimilated into the algae and excreted from the algae as extracellular products were measured at three hours exposure by liquid scintillation counter(Searl Mark III) after removing free $^{14}CO_2$. (Fig. 3)

The results were shown in Fig.4, 5, 6 and 7. The EC_{50} value for three hours exposure might be estimated from the graphs obtained by plotting the percentage of $^{14}CO_2$ assimilated in sample laver exposed to the chemicals or effluents in a certain range of concentration or dilution to the control. Obtained EC_{50} were 4.0 mg/l and 0.01 mg/l for PCP and DCMU respectively. Those for C12 LAS and sewage treatment effluent were not obtained in significant EC_{50} value. Thus, the inhibitory effect of substances to photosynthetic activity of Porphyra yezoensis could be sufficiently observed by this method.

Further more, some examples observed growth of Porphyra

yezoensis being exposed to an effluent in different dilution ratio and salinity were demonstrated as shown in Fig. 8.

III. Summary

(I) Biomonitoring

(1) Continuous biomonitoring system are favourable to avoid unexpected risk to give a damage to ecosystem.

(2) Cost of hardware for the system and maintenance are not expensive.

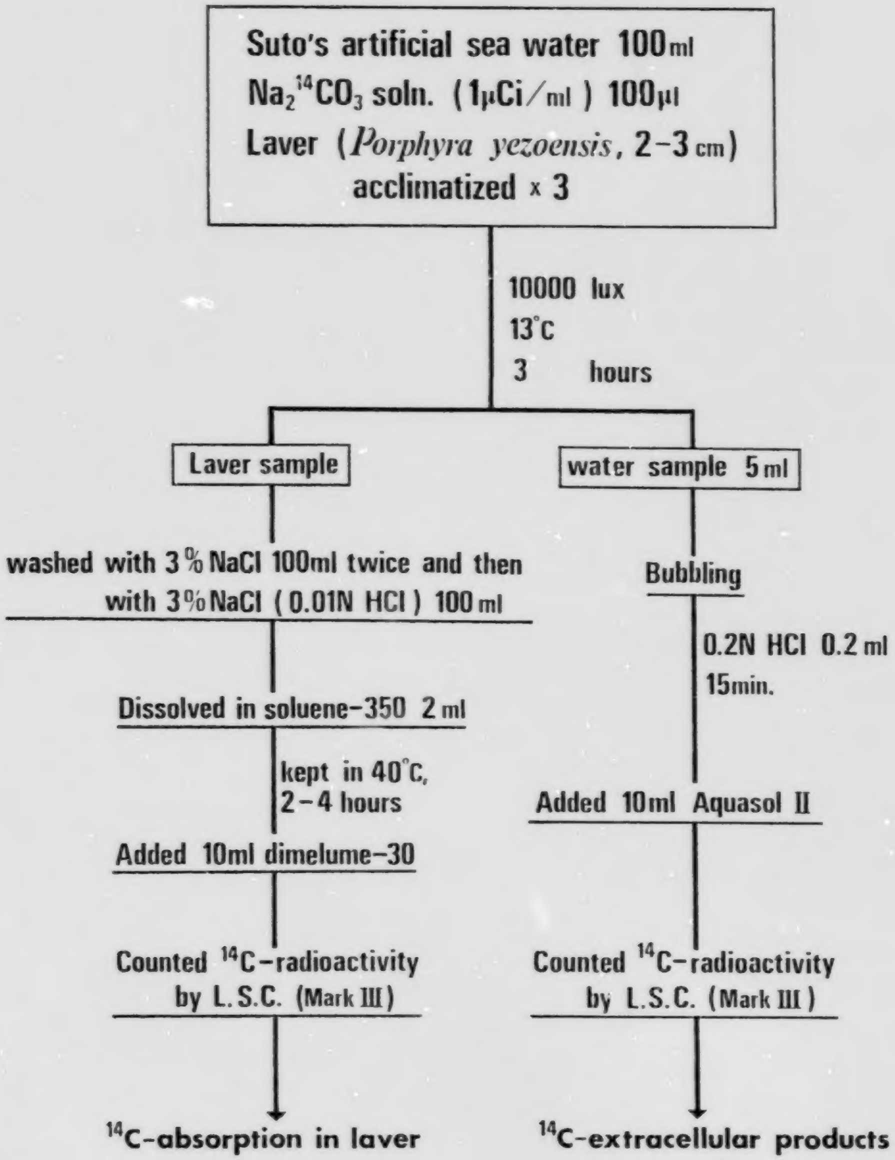
(II) *Porphyra sp.* as biomonitoring specimen

(1) *Porphyra sp.* is considered favourable test species for evaluation of hazardous effects of chemicals as well as treated effluent, although there are much remained to finalized the procedure.

(2) Effects may be found by measurements of growth, photosynthetic activity, constituents, and diseases.

(3) Whenever, the specimen of any life stage can be easily obtained.

Fig. 3 Measurement of photosynthetic activity


Suto's artificial sea water 100ml
Na$_2$^{14}CO$_3$ soln. (1μCi/ml) 100μl
Laver (*Porphyra yezoensis*, 2-3 cm)
acclimatized x 3
10000 lux
13°C
3 hours
Laver sample
water sample 5ml
washed with 3% NaCl 100ml twice and then with 3% NaCl (0.01N HCl) 100 ml
Bubbling
0.2N HCl 0.2 ml
15min.
Dissolved in soluene-350 2 ml
kept in 40°C, 2-4 hours
Added 10ml Aquasol II
Added 10ml dimelume-30
Counted ^{14}C-radioactivity by L.S.C. (Mark III)
Counted ^{14}C-radioactivity by L.S.C. (Mark III)
^{14}C-absorption in laver
^{14}C-extracellular products

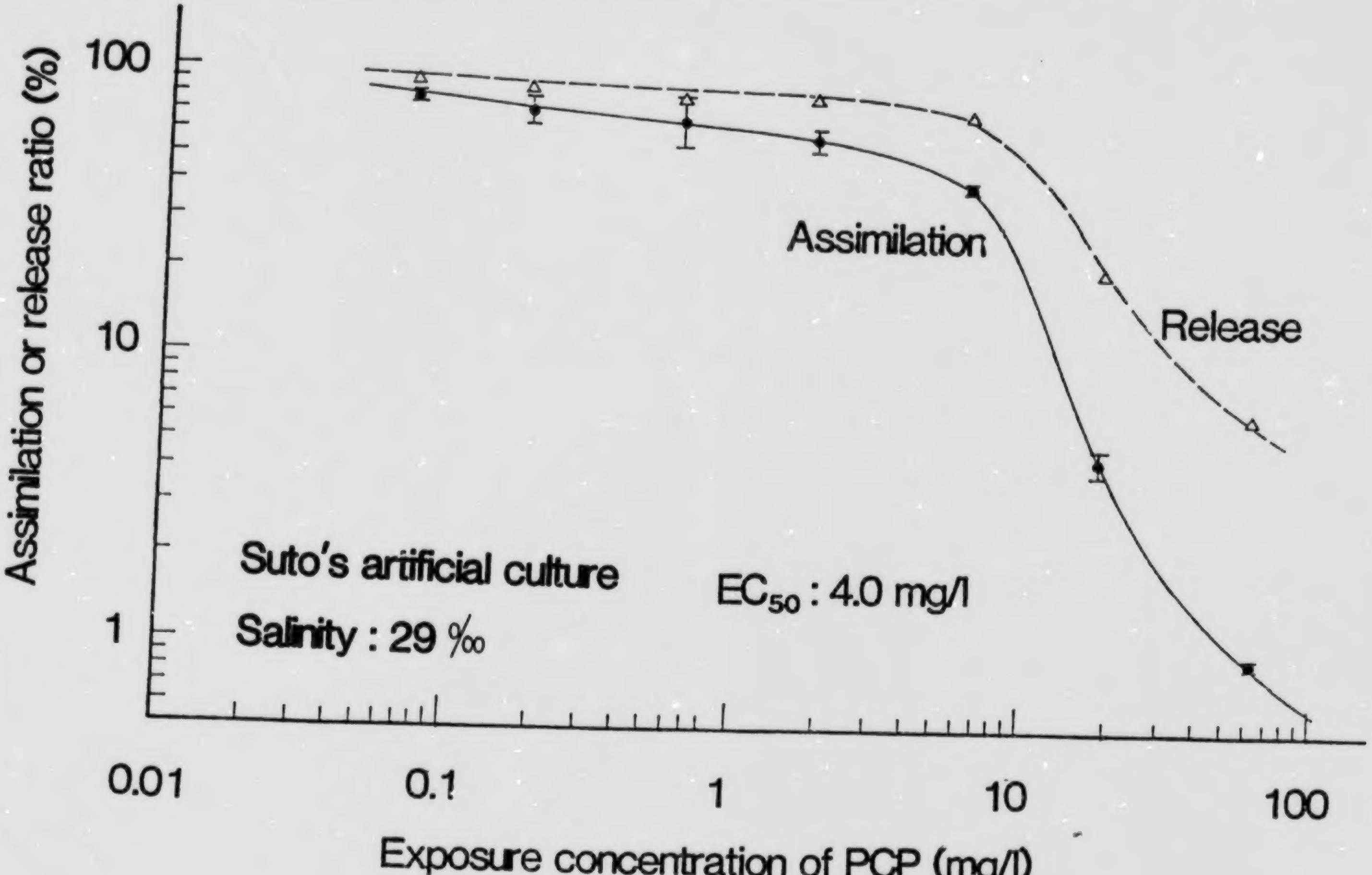


Fig. 4 Inhibitory effect on photosynthetic activityin laver (*Porphyra yezoensis*) exposed to different concentration of Pentachlorophenol (PCP) with assimilation of $^{14}CO_2$ and its extracelluler release (3 hours exposur).

—●— Assimilation ratio: Assimilated ^{14}C compare with control

--△-- Release ratio: Released ^{14}C-organic substance compare with control

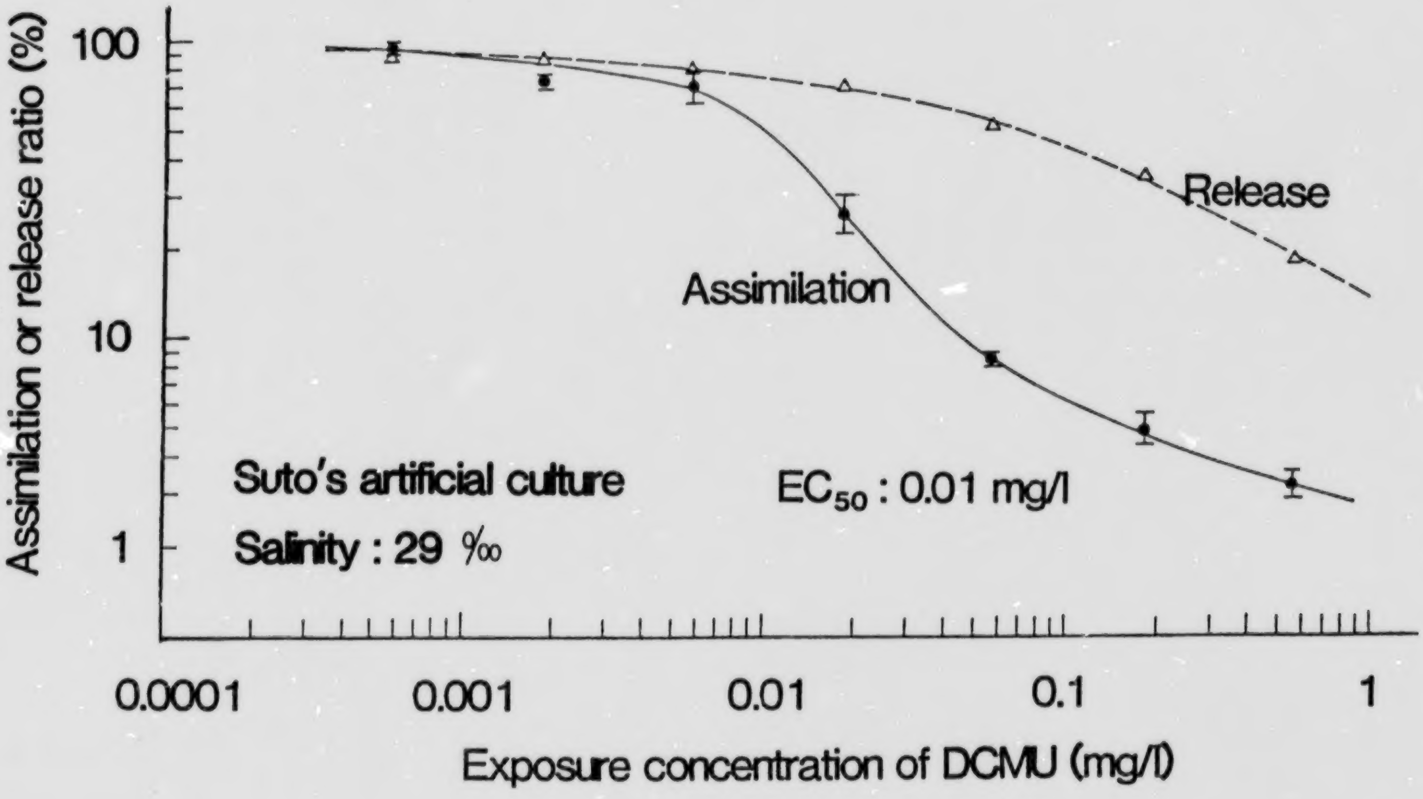


Fig. 5 Inhibitory effect on photosynthetic activity laver (*Porphyra yezoensis*) exposed to different concentration of 3-(3,4-dichlorophenyl)-1,1-demethylurea (DCMU) with assimilation of $^{14}CO_2$ and its extracelluler release (3 hours exposur).

——●—— Assimilation ratio: Assimilated ^{14}C compare with control

– –△– – Release ratio: Released ^{14}C-organic substance compare with control

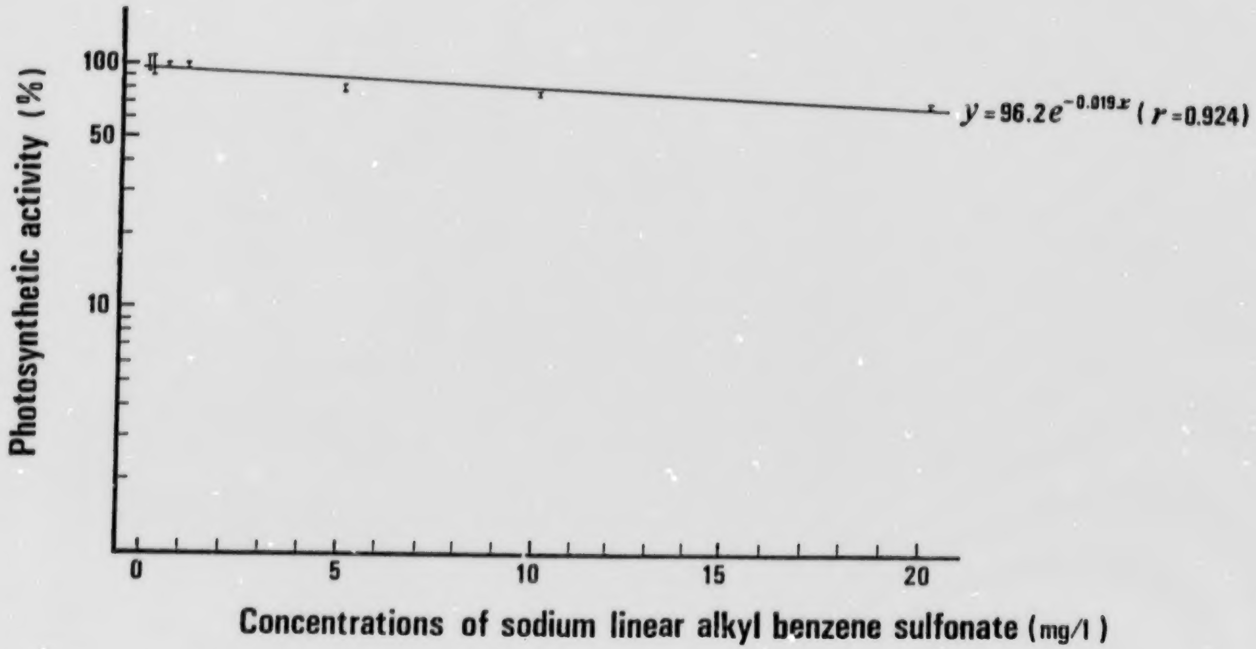


Fig. 6 Inhibition rate of photosynthetic activity in laver exposed to different concentrations of sodium linear alkyl benzene sulfonate (3 hours exposur)

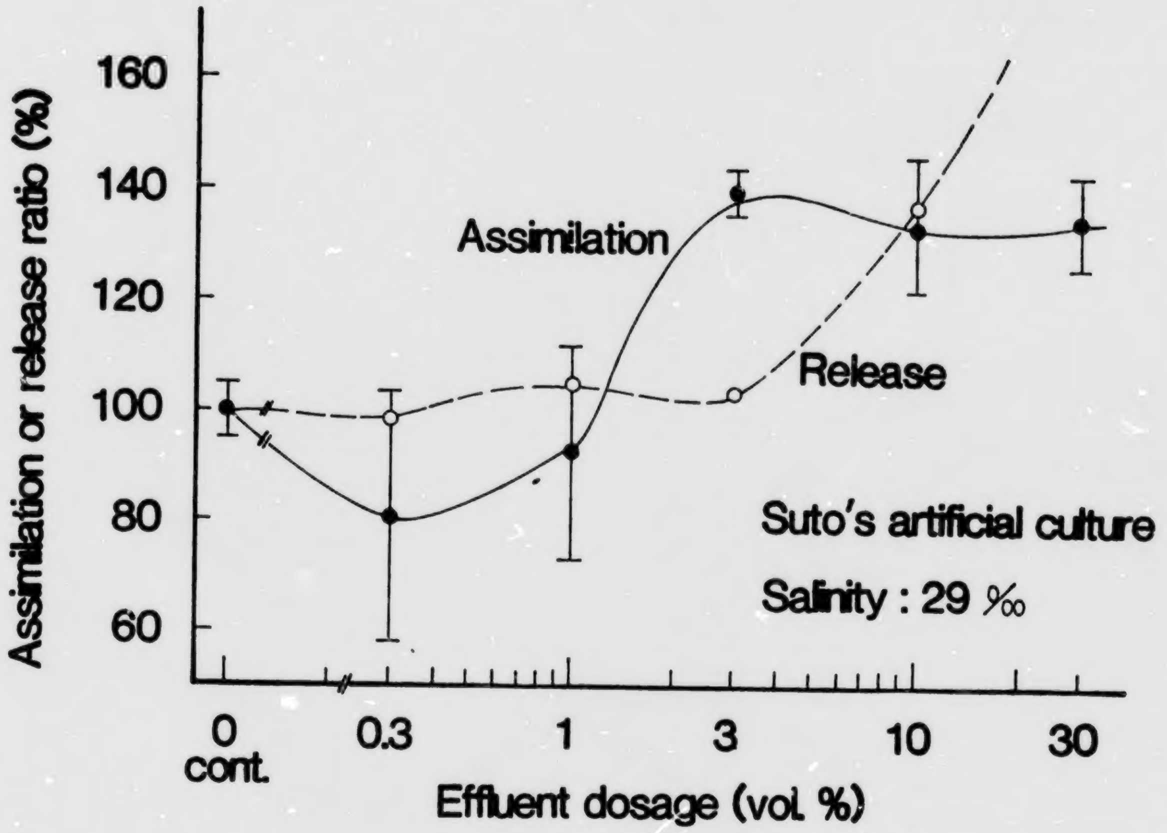


Fig. 7 Effects on photosynthetic activity of laver, *Porphyra yezoensis*, exposed to different content of the treated municipal effluent with $^{14}CO_2$ assimilation and rerease of extracelluler substances (3 hours exposur).

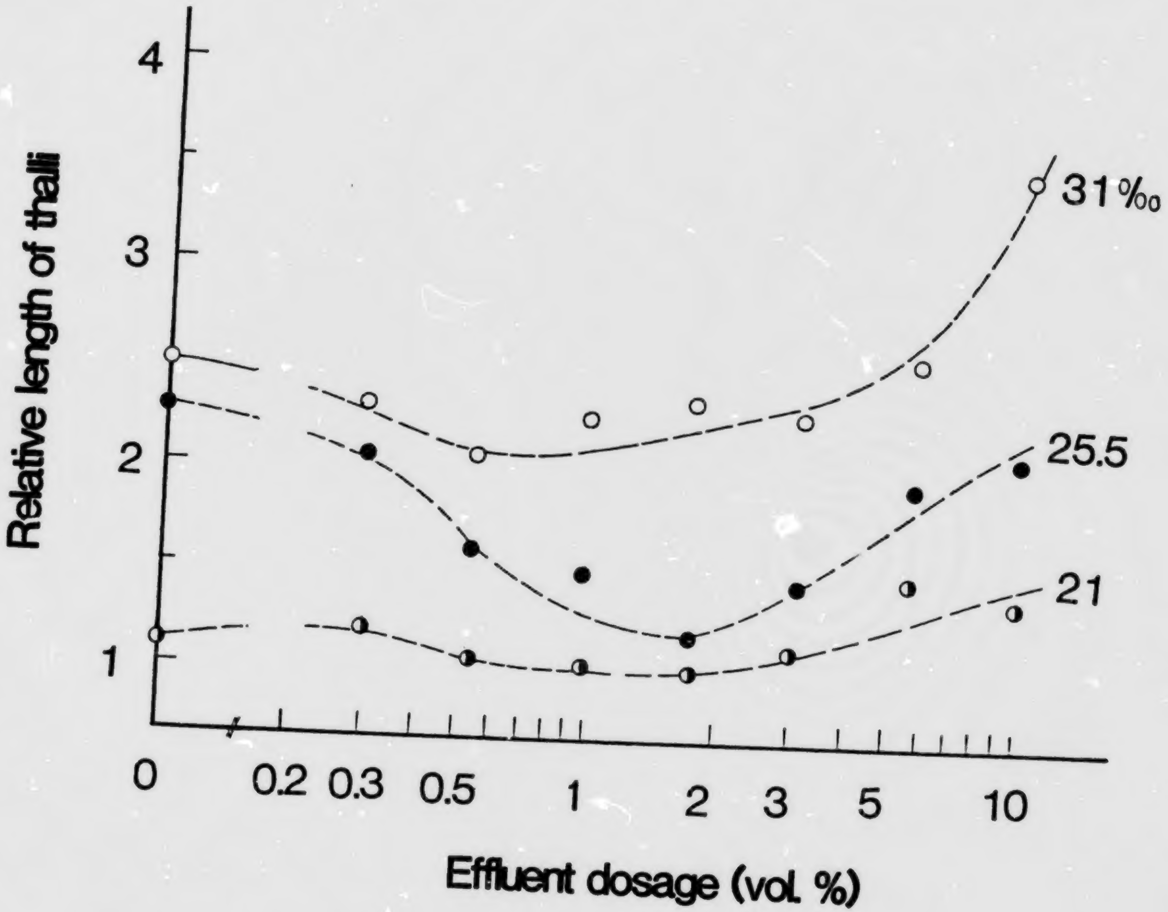


Fig. 8 Effects of treated municipal sewage effluent on relative length of thalli of *Porphyra yezoensis* as a function of effluent dosage for different salinities after 12-days shaking culture.

Biological Monitoring of Water in the Federal Republic of Germany Regulatory Approaches and Methods for Effluent Testing and Assessment of Receiving Waters

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Introduction

The guiding principle for regulatory decisions by the German Federal and State Water Management Administration is to cancel out any avoidable impact and to keep any risks to the environment at a minimum by opting for the best technology available. The technological regulatory approach made mandatory by the Federal Water Management Act (Wasserhaushaltsgesetz) is to be applied even under conditions where it is arguable whether it might lead to "overprotection" of the specific environment involved. Another principle implies that ocean disposal or dumping of wastes as an alternative to an effective treatment of industrial wastes and land-based deposition of sludges and other potentially toxic waste products is acceptable only under exceptional circumstances and not as a long-term strategy.

Apart from the technological approach to keep man-made effects on the aquatic environment at a minimum by formulating uniform emission standards (UES), environmental quality objectives (EQO) are used as a second instrument in the German Water Management policy. Only by reasonably combining both approaches, each supporting the other, can an effective management of the aquatic resources be achieved, specifically under those conditions where even the best available technology does not meet the requirements regarding the water quality standards agreed on.

TABLE I. GENERAL BIOLOGICAL MONITORING

INLAND FLOWING WATERS

BIOCENOTIC CRITERIA DEFINED BY LAWA (1976)

SAPROBIC INDEX

TYPE OF COMMUNITY METABOLISM : AUTOTROPHIC VS HETEROTROPHIC ACTIVITIES

INLAND LAKES (NO GENERAL CONSENSUS ON METHODS)

STATUS OF EUTROPHICATION

BIOCENOTIC CRITERIA

TYPE OF COMMUNITY METABOLISM : AUTOTROPHIC VS HETEROTROPHIC ACTIVITIES

ESTUARINE AND MARINE WATERS (NO GENERAL CONSENSUS ON METHODS)

BIOCENOTIC CRITERIA (LONG-TERM TIME-SERIES OF PHYTOPLANKTON AND ZOOBENTHOS)

TYPE OF COMMUNITY METABOLISM : AUTOTROPHIC VS HETEROTROPHIC ACTIVITIES

General Biological Monitoring of Inland and Marine Waters

Table I

The general monitoring of the water quality in inland waters is conducted by applying biological criteria agreed on by all German State Water Management administrations. Biological criteria for the classification of estuarineand marine waters are under discussion. Criteria primarily involve community structures of both phytoplankton and zooplankton, and phytobenthos and zoobenthos, but preferentially of the macroinvertebrates. The use of the Kolkwitz/Marsson system of indicator species and the calculation of a Saprobic Index is restricted to flwoing inland waters. Generally, criteria are used whereintensities and relationships of the autotrophic and heterotrophic components of the overall community metabolism as well as their effects on the oxygen budget are involved. Results of the biological water quality monitoring of all major German waters are published every fifth year; those on areas of specific interest every year.

Health Status of Fish Populations

Table II

In inland as well as in marine waters the health status of fish populations is investigated. Dependences of fish diseases on effects of pollution are a matter of controverse discussion especially in the estarine and marine environment.

Monitoring Environmental Toxicity in Receiving Waters

Tables III to IV

Bioassay techniques have been developed and have been applied successfully for both, measuring the toxicity of water samples retrieved from polluted waters, as for continuously instream monitoring of water toxicity. Exemples are the case studies on the Lower Wupper (Knie, 1983) a tributary of the river Rhine, and on waters within the Hamburg community (Dannenberg, 1982). The Lower Wupper could be sectioned into several

Table II. Fish diseases investigated in the German Bight and other areas of the North Sea (Dethlefsen)

Dab	Cod
Lymphocystis	Pseudobranchial tumours
Ulceration	Ulceration
Epiderman papilloma	Skeletal deformations
Hyperplasia	Fin rot
Skeletal deformations	and others
Pigment anomalies	
Fin rot	
and others	

Table III, Toxicity of receiving waters to *Daphnia magna* according to Knie (1983)

Dilution to obtain a NOEL	Classes of water toxicity
0	NON TOXIC
0 - 2	SLIGHTLY TOXIC
2 - 6	CRITICALLY TOXIC
6 - 12	HIGHLY TOXIC
MORE THAN 12	EXCESSIVELY TOXIC

TABLE IV. TOXICITY OF RECEIVING WATERS TO HYDRA LITTORALIS

INHIBITION OF REPRODUCTION	CLASSES OF WATER TOXICITY	(PROVISIONAL)
0 - 1 %	NOT OBSERVABLY TOXIC	
2 - 6 %	SLIGHTLY TOXIC	(SUBCHRONICALLY SUBLETHAL)
7 - 15 %	CRITICALLY TOXIC	(SUBCHRONICALLY SUBLETHAL)
16 - 30 %	HIGHLY TOXIC	(SUBCHRONICALLY SUBLETHAL)
31 - 60 %	HIGHLY TOXIC	(SUBCHRONICALLY LETHAL)
61 - 90 %	EXCESSIVELY TOXIC	(SUBCHRONICALLY LETHAL)
91 - 100 %	EXCESSIVELY TOXIC	(ACUTE LETHAL)

classes of toxicity leves by investigating of water samples by means of the static Daphnia test. For waters in the Hamburg area, the hydroid bioassay successfully applied.

Methods for effects monitoring have been also developed for the application in marine waters by using various test organisms. However, results obtained from studies in different areas of the North Sea are not as clear as those from limnic waters.

The results of the work concluded so far show that there is no single bioassay using one species of test organisms as a standard toxicity test for toxicological effects monitoring that could be recommended for general application. It depends on the site-specific, ecotoxicological situation of the water-receiving effluent contaminated by pollutants that are especially harmful to specific taxa of water organisms whether preference is given to the use of one species of test organisms or another. Having this in mind, the authorities are either planning or have installed test batteries that combine different static and/or dynamic testprocedures. At present, preference is given to a combined algae-Daphnia-fish bioassay.

The same method of using a combination of different bioassays is also used for continuous instream toxicity testing. A case in point are the two water pollution control stations on the Rhine river managed by the State of Nordrhine-Westphalia: one upstream station at Bad Honnef (km 64o bordering Rhineland-Palatinate) and one downstream station at Kleve/ Bimmen (km 865 at the German/Dutch border).

At these two stations toxicity flow-through testing batteries are being installed combining two dynamic tests: a fish test (Leuciscus idus, swimming behaviour) and a crustacean test (Daphnia magna, swimming behaviour). Both test are specifically designed as in-stream, early warning tests as a protection measure for the water works dependent on the waters of the Rhine system. An additional discontinuous oxygen production of water samples is measured every day using the light-and--dark-bottle technique(Determination of the potential oxygen production under standardized laboratory conditions. The oxygen production is related to the chlorophyll content of the samples.

Biological Monitoring of Specific Water Pollutants

Biological instream monitoring of specific water pollutants is conducted through exposure of caged animals (and plants) which are then analysed for anorganic and organic trace contaminants several times a year. In addition to the contaminants' body burden the changes in the biochemical condition of the exposed organisms are investigated as they are essential for the interpretation of seasonal and regional differences in concentration of contaminants accumulated by the organisms. Preference is given to the use of mussels as biological accumulators (The blue mussel, Mytilus edulis in marine, the zebra mussel, Dreissena polymorpha, in limnic waters).

Effluent Monitoring

The same approach as to receiving waters is used or is planned to be used for biological effluent monitoring and endpipe control of wastes to be discharged into receiving waters within the limitations by the licensing authorities. However, up to now, the realisation of an effective system for a continuous toxicity control is a far way off. Problems arise specifically with respect to the numerous medium- and small-size industries discharging treated and untreated wastes directly into the receiving waters.

For all effluents, a repeated testing by way of a static fish test is mandatory according to the Effluents Taxation Act.

Most of the major dischargers are requested to use a dynamic flow through fish test for a continuous control of their treated effluents. For this purpose it is usually allowed for the effluents to be diluted by defined dilution rates using tap water or water taken from the receiving water system. In many cases, the effluents are additionally tested discontinuously, every day or one a week throug various bioassay techniques. Preferentially, the static Daphnia test according to OECD guideline 2o2 Part 1 is used.

There is also some discussion among the authorities whether in the case of effluent monitoring only batteries that combine different dynamic bioassay techniques can meet the needs for an effective biological effluents control. However, costs and the lack of the much needed qualified personnel may keep this approach from being realized in the near future.

General Conclusions

- There is a need for a more general application of a continuous toxicity testing in effluent control.
- Only a combination of different bioassay techniques integrating the responses of 3 to 4 types of test organisms differing in sensitivity to a broad spectrum of pollutants can be effective.
- The same types of batteries should be used for monitoring effluents and receiving waters.
- Suitable techniques have to be selected according to their practicability, sensitivity and reproducibility and also according to their interpretability with respect to the objectives of water management policies.

BIOLOGICAL TESTING OF EFFLUENTS AND SOLID WASTES IN SPAIN

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ABSTRACT

The Bioassay Techniques used by three Spanish organizations as well as the problems found will be described.
The three organizations are located in Catalonia, since this region is the only one in Spain where the Bioassay techniques are used for deriving discharge limitations. Research has been carried out in some Spanish Universities about the microorganisms present in polluted rivers, but the results have not been so far applied to establish control priorities or deriving dischargege limitations.

The organizations and the Bioassay techniques used are the following:

- Environmental Services, Local Government of Catalonia.

  A stepwise strategy for toxicity evaluation of industrial solid wastes to set the suitability of land fill disposal treatment, has been developed. This strategy relies on the desing of a standard test for lixiviation of wastes and subsequent evaluation of toxicity.
  A systematic comparison between Daphnia Magna test and a instrumental bioassay (with Photobacterium Phosphoreum) has been done.

- Institute of Fishery Research (National Research Council)

  Ecotoxicity of 93 products and wastewater from a pharmaceutical industry whose effluents are discharged into the sea have been controlled with regard to the behaviour of Daphnia Magna and Artemia Salina shell fish and the Mugil Auratus fish.

  A comparative study of the response of Daphnia Magna and Artemia Salina shell fish together with that of sea plankton Temora Stylifera, against some surfactants, has been carried out.

- Department of Pollution Control of Intextar, Polythecnic Universiti of Catalonia.

  Rainbow trout and Brachydanio Rerio fish, Daphnia Magna shell fish the Chlamydomonas Variabilis algae and bacterias are used in the Bioassay tests performed on wastewater and chemicals used by the textile industry.

  Research is being presently carried out on bioaccumulation and the physiologycal effects caused by metal complex dyes on the Rainbow trout.

The Bioassay Techniques used by three Spanish organizations as well as the problems found will be described.

The three organizations are located in Catalonia, since this region is the only one in Spain where the Bioassay techniques are used for deriving discharge limitations. Research has been carried out in some Spanish Universities about the microorganisms present in polluted rivers, but the results have not been so far applied to establish control priorities or deriving discharge limitations.

The organizations and the Bioassay techniques used are the following:

ENVIRONMENTAL SERVICES, LOCAL GOVERNMENT OF CATALONIA.

Introduction.

The regional Government of Catalonia has enacted a Law on Industrial Wastes that tries to solve the final disposal problem of wastes coming from the Catalan Industry.

Catalonia, 32000 Km^2, 6000000 people, the first industrialized Region in Spain, faces an important problem of waste disposal facilities. (Estimated overall production: 2.000.000 tons/year).

In fact, 75% of industrial wastes are produced in the industrial area of Barcelona, scarcely 500 Km^2.

The Law establishes that full responsibility of industrial waste disposal relies on the producer, and sets guidelines for industrial waste characterization.

So, a stepwise characterization system has been developed on behalf of Environmental Service of the Ministry of Public Works and Land Planning (DEPARTAMENT DE POLITICA TERRITORIAL I OBRES PUBLIQUES, GENERALITAT DE CATALUNYA), in order to make clear a standard legal procedure for waste classification.

The work has been carried out by Mrs. Asunción Calleja, from Laboratori General d'Assaigs i Investigacions (LGEI), and Dr. Martín Crespi, from Institut Textil de Terrassa, both teams coordinated by Dr. José M$\underline{^{a}}$ Baldasano, from Servei de Medi Ambient.

The results of that research have allowed to settle a legal procedure to identify and classify the industrial wastes produced in Catalonia. It is on stream since June 84.

Lixiviation test.

The first step of the overall procedure has been the settling of standard conditions for a lixiviation test.

It is known that the most important effect of industrial wastes disposed on a landfill facility relies on lixiviates. A lixiviation test, carried out in laboratory in a reproducible way and allowing the characterization of pollutants present in the lixiviation waters, is of extreme importance.

From the comparison of the different known tests,(13) (14) (8), which are discussed in the Table 1, a batch test has been developed, in which various parameters governing the phenomena were explored by means of a factorial experimental design.

Table 1.- Comparison of lixiviation tests.

1 months

TYPE	ADVANTAGES	DISADVANTAGES
Reduced Scale Model 13	- Simulates reality - Includes environmental factors - The kinetics can be studied	- Expensive - Very time-consuming -It is specific of the site
Column Type (13),(14), (8)	- Similar to reality - Kinetics can be studied - Simple, minimum equipm. - Low cost	- Very time-consuming - Difficulties in getting a uniform package - Column clogging - Biological activity - Wall effects - Low reproductibility
Batch Type (13), (14), (8)	- Simple, minimum equipm. - Low cost - Quick - Good reproducibility	- Does not include environmental factors - Kinetics cannot be studied

With 4 parameters, 2 levels each, for every waste, optimal conditions have been evaluated for 5 types of wastes, namely:

1) Pesticides containing Malathion Chlorpropham, Propham, Bendiocarb, MethylAzinfos.

2) Waste coming form potassium dichromate manufacturing.

3) Sludges from neutralization of metal surface industry effluents.

4) The same sludge after an inertization process.

5) Acid sludges from a lubricants recovery industry.

Two of parameters, - type of lixiviating agent and relation liquid/solid -, have relevance on results, being lixiviation time and temperature of minor importance.

From these results, a proposal for the lixiviation test has been set up.

Bioassay Test.

On lixiviates coming from the previous experiences, a comparison between two bioassays has been carried out. Because of Daphnia Magna toxicity test is, among other reasons, rather involving a quicker test has been searched.

An instrumental luminiscent bioassay, by means of Photobacterium Phosphoreum has been compared with Daphnia Magna.

Photobacterium Phosphoreum test was already compared (5) with S. volutans, P.fluorescens, and A. Hydrophila, giving excellent results.

The traditional difficulty in bioassays, that is toxicity, -it depends on organism nature, being therefore impossible to establish an absolute value of toxicity -, has been faced trying to set the toxicity range equivalence between both tests, (Daphnia and Photobacterium), following some promising results of Kenaga (12).

A similar method of that proposed by Bulich (3), has been used.

For Daphnia Magna, a CI 50 in 24 hours has been measured, for Photobacterium Phosphoreum (through Microtox) and EC 50 in 5 minutes, 15 minutes and 30 minutes has been evaluated.

Four different criteria have been used for test comparison, the three of Bulich (3) and one of Servei de Medi Ambient, Generalitat de Catalunya.

A. Classification according the toxic-non toxic concept.

Table 2. Comparison for 37 samples (industrial waste lixiviates as described.

		LGEI	(Bulich)
Criteria 25%			
D.M. and Microtox, toxic 29/37 :	78,4%	Agreement	
D.M. and Microtox, non toxic 2/37 :	5,4%	83.8%	(85%)
D.M. toxic, Microtox non toxic 5/37:	13.5%	Disagreement	
D.M. non toxic, Microtox toxic 1/37:	2.7%	16,2%	(15%)
Criteria 50%			
D.M. and Microtox, toxic 32/37 :	86,5%	Agreement	
D.M. and Microtox, non toxic 0/37 :	0 %	86.5%	(75%)
D.M. toxic,Microtox non toxic 4/37 :	10,8%	Disagreement	
D.M. non toxic,Microtox toxic 1/37 :	2,7%	13,5%	(25%)

B. Classification according percentages.

From the table that classifies categories according to results of the specific bioassay:

EC/CI 50	Class	Category
25%	Very toxic	1
25 - 75%	Toxic	2
75%	Fairly toxic	3
Non-toxic	Non toxic	4

and for the mentioned 37 samples, the degree of agreement is shown in Table 3.

Table 3

Daphnia Magna and Microtox		LGEI	(Bulich)
Samples assigned to the same category	31	83,8%	(63%)
Samples assigned with one degree diff.	6	16,2%	(10%)
Samples assigned with two degrees dif.	0	0 %	(17%)
Samples assigned with three deg, diff.	0	0 %	(10%)

C. Classification according logarithm category.

The results are shown in Table 4.

Table 4

Daphnia Magna and Microtox, results with a logarithm difference

		LGEI	(Bulich)	(Kenaga)
less than 0,5	24/37	64,9%	(50%)	(-)
less than 1,0	29/37	78,4%	(80,1%)	(-)
less than 1,5	34/37	91,9%	(96,7%)	(95%)
less than 2,0	37/37	100 %	(98,7%)	(-)
mean of log. differences		0,46	(0,34)	(0,49)

D. Classification according criteria of Servei de Medi Ambient.

These criteria settles that a waste is "special", -that is, its final disposal must be done in a special place or facility previously approved -, when its lixiviates, obtained by the standard lixiviation test, have a CI 50 $\leq$ 750 mg/l for Daphnia Magna 24 hours, or a EC 50$\leq$ 3000 mg/l for Photobacterium Phosphoreum (through Microtox).

For the previous 37 samples and a EC 50 in 5 minutes, the results are those of Table 5.

Table 5

D.M. and Microtox, toxic 15/37	: 40,5%	Agreement
D.M. and Microtox, non toxic 14/37	: 37,8%	78,3%
D.M. toxic, Microtox non toxic 5/37	: 13,5%	Disagreement
D.M. non toxic,Microtox toxic 3/37	: 8,1%	21,6%

Conclusions

The results shown here have decided us to go on implementing the test by legal procedures. We are conscious that this is a first attempt to waste characterization of toxicity, but it has proved very useful in the cases we have been involved recently as a Regional Administration.

We foresee difficulties in defining a precise borderline between what is acceptable as toxic and what is not, but are aware also that environment and nature do not behave with such precise limits . Nevertheless we expect further progress in that field.

INSTITUTE OF FISHERY RESEARCH

(National Research Council)

Acute toxicity of 93 chemical products and wastewaters from a pharmaceutical industry whose effluents are discharged into the sea have been controlled with regard to the behaviour of Daphnia Magna and Artemia Salina Shellfish and the Mugil Auratus fish, on request of the Local Government of Catalonia. Probits method (15) has been used for the computation or results. In all cases, Artemia Salina has proved much more resistant than Daphnia Magna; on the other hand, the Mugil Auratus fish was found to be very sensitive to pH, which made it necessary to neutralize the samples.

A comparative study of the response of Daphnia Magna and Artemia Salina Shellfish together with that of sea plankton Temora Stylifera, against linear alkyl sulfate, nonylphenol aethoxylat, lauril benzyl - bis (β -oxyäthyl) ammonium chloride, potassium dichromate and products from the biodegradation of dodecylbenzene sodium sulphate has been carried out. Generally speaking, (Artemia Salina is more resistant to the products tried than the rest of the species considered . However, Daphnias are more resistant than the other species versus the Linear Alkyl Sulfat. Another exception is the Temora Stylifera which, in spite of being the most sensitive organism for the surfactants tried, gives up this condition in favour of the Daphnia Magna versus the Lauryl-Benzyl-bis (β - oxyäthyl) Ammonium Chloride.

The resulting products from the biodegradation of dodecyl-bencene-sodium sulfonate are not toxic for the Artemia Salina; it has also been observed to exist a close relation between the decreasing values of the surfactants and the progressive decrease of their acute toxicity. Therefore, the biodegradation processes of surfactants can be known through their immobilizing action on the organisms sensitive to them.

DEPARTMENT OF POLLUTION CONTROL OF INTEXTAR

(Polytechnic University of Catalonia)

The acute toxicity of both wastewater and lixiviates of industrial solid wastes is determined by the inhibition of the mobility of Daphnia Magna Straus (9), since this is the acute bioassay approved by the Act 5/1981 of the local Government of Catalonia to fix the amount to be paid for discharging wastewaters and to establish control priorities.

This bioassay has also been used to determine the decrease of toxicity on applying different biological and physicochemical depuration processes on textile wastewaters (4). The highest decrease of toxicity was achieved by an activated sludge process.

Other bioassays are usually carried out on chemicals, mainly dyestuffs, made in Spain and exported to Europe, such as: Acute toxicity in Rainbow trout (6), Inhibitory effect of a chemical substance on aerobic wastewater bacteria (7), biodegradation tests (16).

These bioassays have to be made as the European legislation demands its fulfillment in the European Core Inventory (ECOIN).

Ocasionally, the Brachydanio Rerio fish (2) and the Chlamydomonas Variabilis algae (11) have been used in the Bioassay tests.

Research is being presently carried out on bioaccumulation and the physcological effects caused by metal complex dyes with cobalt, chrome, copper or nickel in its molecule on the rainbow trout. This work will determine the acute and subchronic toxicities and the bioaccumulation of four premetallized dyes with the same azoic base and different metal on the branchiae, liver, kidney, pancreas and muscles of the rainbow trout. The acute and subchronic toxicities of the above dyes will be performed on trouts in different growing periods.

The effects caused by the mentioned metal complex dyes on the cardiorespiratory system in adult fish and on the nervous system is examined by studying the colinesterasic activity in the brain and skeleton muscle during the different growing periods.

General Conclusions.

1. Catalonia is the only one region in Spain where the Bioassay techniques are used for deriving discharge limitations. For legal effects, the acute toxicity test carried out is that of Daphnia Magna (9), Act 5/1981 of the Local Government of Catalonia.

2. In the acute toxicity bioassays performed we have found a very ample response according to the organisms used, which leads to a lack of agreement on the application of results and makes difficult the comparison between tests.

3. It would be most necessary to design an acute toxicity bioassay with a wide application range and easy to perform, which could be used as a routine test even in non-specialized laboratories.

4. A toxicity bioassay, internationally accepted, should be developed in order to facilitate the comparison of results.

5. It is necessary to find new techniques, less complicated and more reliable than the present ones to predict the long term effects.

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SHORT OVERVIEW OF BIOLOGICAL MONITORING OF EFFLUENTS AND RELATED RECEIVING WATERS IN NORWAY

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Abstract

The Norwegian State Pollution Control Authority (SFT) uses results from biological monitoring in administrative decisions concerning important pollution control measures.

In Norway there is a legal basis for conducting biological assessment through the Pollution Control Act.

The most frequent use of biological monitoring in Norway is the monitoring of receiving waters.

Biological testing on the whole effluent from an industrial discharger has sometimes been accomplished. An example of such testing is included.

In the future promising biological monitoring methods and techniques should be given priority, as well as cause/effect studies.

Introduction

The Norwegian State Pollution Control Authority (SFT) uses results from biological monitoring in administrative decisions concerning important pollution control measures.

The reason for using biological monitoring when making decisions on the control of water pollution is that it supports and strengthens the results obtained from other monitoring activities, especially from chemical monitoring and control. It can also provide guidance for more comprehensive physical/chemical investigations, as well as indicating where and when it is necessary to carry out more intensive surveys.

Legal basis

Through the Pollution Control Act which entered into force in October 1983 there is a legal basis in Norway for conducting biological assessment, both for deriving water quality standards and discharge limitations.

Different areas for using biological monitoring

The most frequent use of biological monitoring in Norway is the monitoring of receiving waters. Surveys are mainly conducted as part of the national monitoring programme both in marine and fresh water, but also in connection with hydroelectric power schemes, wastewater discharge, eutrophication problems etc. In these surveys in situ monitoring of the natural biological communities prevail, but bioassays are sometimes included. Cause/effect studies are also carried out experimentally in marine littoral and benthic communities where effects of low concentrations of oil are investigated. In addition we refer to the Norwegian paper submitted to this workshop concerning the correlation between pollutant load and the diversity of marine soft-bottom fauna communities.

On the other hand, for regulatory monitoring purposes biological monitoring is not so much used, but we expect this will change. A project with the purpose of introducing the use of ecotoxicological tests in regulation has been suggested.

A program of ecotoxicological tests has been set up as a basis for licensing use of the different drilling-muds used in the offshore oil industri. In this connection a program has also been established for the testing of dispersants for which permission for use is considered by the authorities.

As regarding research activities, work has been centered on development and assessment of bioassay methods for bacteria, protozoa, algae, invertebrates and fish. The effect of metal ions on fish has been investigated in acute and chronic tests. Bioassays has also been an important tool in the study of the effects of acid rain. The influence of water quality parameters on the uptake and toxicity of micropollutants has been investigated. In the future we would like to improve the predictive value of bioassays by the development of methods, and by gaining more experience from simple bioassays, controlled ecosystem experiments and case studies.

Biological testing on the whole effluent from an industrial discharger has sometimes been accomplished. These tests are done to get a first indication of the effluent quality as regards biological effects. Usually a set of acute bioassays (toxicity tests) are used (algae, daphnia, fish), together with tests of bioaccumulation and degradability. In situ exposure of fish in cages has also been used. The tests are generally not used for regulatory purpose. However, an example of such testing can be mentioned.

An example

The Nitroglycerine Plant (Dyno Industries A/S) is planning to expand its production of ammunition. This will cause an increase in the discharges to the inner part of the Oslo-fjord, which receives the domestic discharges from about 600.000 p.e. On this background the Norwegian State Pollution Control Authority required that the company should perform a test program on their effluents.

It was believed that some of the nitroaromatic compounds in the effluent could cause toxic effects in the receiving water. The laboratory tests included toxicity tests with different amounts of effluent water mixed with natural or artificial sea water. It was tested on reduced growth rate of two algae, the biodegradability by heterotrophic microorganisms, movements of a crustacea (Artemia-nauplii), and acute toxicity to fish (Gadus morhua juv.). The biodegradability was tested in a 28 days BOD experiment. The mutagenic activity was tested with an Ames test.

From these results and the knowledge about the receiving water, it has been concluded that concerning acute toxicity the receiving water could presumably tolerate the increased discharge of polluted water. To prevent any serious damage to the marine environment the industry will, however, have to set up a program for biological monitoring in the vicinity of the discharge area.

Future work

In the future promising biological monitoring methods and techniques should be given priority, as well as cause/effect studies. An important area is also the development of standard tests. The work which will be done in the OECD project ought to be continued to emphasize the need for using biological testing in the decision-making process. It should also be taken into account that the overall goal of biological testing is to unveil damage on the environment.

CORRELATION BETWEEN POLLUTANT LOAD AND THE DIVERSITY OF MARINE SOFT-BOTTOM FAUNA COMMUNITIES

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Abstract

The soft-bottom fauna are the animal communities living on and within bottom sediments. The density of individuals is frequently between 1000 and 2000 and the number of species between 60 and 90 per m^2 in fjords and coastal waters. The distribution of individuals among species often tends towards a log-normal or a log series, with few species having high numbers of individuals and many species having fewer or just one individual per sample. With increasing sample size, the number of species rises more slowly than the number of individuals. The diversity curve describes the number of species as a function of the total number of individuals sampled. A high-diversity fauna shows a steeper curve than a low-diversity fauna.

Studies in a number of fjords in Norway showed negative correlation between the diversity of soft-bottom fauna and pollutant discharge amounts, proximity to discharge areas, and sediment pollutant load. An empirically based diversity classification system was developed.

There was a strong negative correlation between fauna diversity and sediment copper concentration. This is explained as a cause-effect correlation, with high copper concentrations being toxic to a number of species. The number of species is roughly halved for each ten times increase in the copper concentration. It is concluded that copper pollution has erased many species in large marine bottom areas in some Norwegian fjords.

The diversity curves may serve as criteria for the ecological effects of pollution and indicate its area of influence. These kinds of biological findings, combined with data on sediment pollutant load and discharge amounts, may form a basis for optimizing environmental protection measures.

Introduction

The industry as well as densely populated areas along the Norwegian coastline are very often situated along fjords, representing well defined point sources of pollution. Fjords are efficient traps of sediments and as the majority of pollutants are associated with particulate matter, the sediments act as a sink [9,10] . Consequently, steep gradients of pollutants occur horisontally as well as vertically in the bottom sediments.

The greater parts of the bottom areas in fjords consist of soft sediments. The surface and sub-surface layers of the sediments are inhabited by animal communities - the soft-bottom fauna. Normally, this fauna is very abundant and diverse. In Norwegian fjords, the number of animals, retained by sieves of 1 mm apertures, typically reaches 1000-2000 per m^2, representing 60-90 different species. The number of individuals among the species makes up the numerical structure of the faunal community. Some species are more abundant than others. Many species have only a few or just one individual per sample. Many species, living at the locality, are not sampled at all due to sample size limitations. The distribution of numbers of individuals among species often tends towards a log-normal or a logarithmic series [7] . Two examples from different localities are shown in Table 1. The map in Figure 1 shows the fjords which are referred to in this paper. Station depths ranged from 20 to 500 metres. None of the stations had anoxic conditions.

By a number of studies, especially in the 1970's it was well documented that pollution often leads to structural changes in the bottom-fauna communities, as revealed by diversity measurements [8]. With increasing pollutant load or other kinds of environmental stress, a few tolerant or oppurtunistic species will become relatively more numerous and will dominate the community, while many less tolerant species will become increasingly rare or disappear altogether. The result will be a lowered diversity.

Methods

Soft-bottom fauna

Samples of the soft-bottom fauna were taken by means of 0.1 m^2 Petersen or Day grabs. The samples were sieved through steel plates perforated

Table 1. The numerical structure of the fauna in samples from two fjords in Norway. The species are ranked by abundance from top to bottom in the table.

Sample A Korsvikfjord 1977, station 2 Number of individuals of each species (n_i)	Sample B Orkdalsfjord 1983, station 7 Number of individuals of each species (n_i)
149	1203
120	1035
82	270
73	123
70	118
63	35
62	16
62	14
39	11
36	10
32	6
24	5
20	2
18	2
17 (x 4 species)	2
13 (x 2)	2
11	1
10	1
9 (x 2)	1
8 (x 2)	1
7 (x 3)	1
5 (x 3)	1
4 (x 4)	1
3 (x 5)	
2 (x 10)	
1 (x 17)	
Number of species (S) = 68	Number of species (S) = 23
Number of individuals (N) = 1103	Number of individuals (N) = 2861

with 1.0 mm diameter round holes [4]. The retained material was sorted in the laboratory.

The distribution of numbers of individuals among species determines the species diversity, which we define as the number of species as a function of the number of individuals. The formula for calculating the diversity curve was given by Hurlbert [5].

$$E(S_n) = \sum_i \left[1 - \frac{\binom{N-N_i}{n}}{\binom{N}{n}} \right]$$

N = the total number of individuals in the sample
N_i = number of individuals of species i
n = the total number of individuals in a sample n/N the size of the main sample
$E(S_n)$ = the expected number of species in a sample n/N the size of the main sample

High species number relative to the number of individuals is equivalent to high species diversity. This gives a steep diversity curve.

The important advantage of this method is that the number of species in samples of different sizes may be directly compared.

The diversity curves for the samples in Table 1 are shown in Figure 2. A logarithmic scale for n is used to obtain a better view of the curves. The sample having the more even distribution of number of individuals among species shows the higher diversity.

With increasing sample size, the number of species increases. However, the number of species increases less rapidly than the number of individuals does, because an increasingly larger proportion of the individuals added will belong to species already found. Thus the steepness of the curve decreases as the sample size becomes larger. In theory we might increase the sample size to the point where all species in the area were represented. The diversity curve would then cease to rise. Figure 3 might represent such an enormous sample.

Sediments

Sediment cores were taken by a gravity corer [6], and sliced in 2 cm sections. Bulk samples were decomposed using conc. HNO_3 and analysed for metals, except the silicious fraction. Polycyclic aromatic hydrocarbons (PAH) were analysed by glasscapillary gaschromatography.

Results and discussion

The diversity of the soft-bottom fauna in some fjords

In the following figures we present some results from studies made by the Norwegian Institute for Water Research in fjords with varying levels of pollution. The investigations were part of the Governmental pollution monitoring program in Norway, financed ty the State Pollution Control Authority.

Figures 4-7 show a number of diversity curves. The end point of a curve is the number of species plotted against the number of individuals in the whole sample. Curves that have similar end points roughly run similar paths. Out of the score of curves, we were therefore able to construct an empirically based classification system (Figures 8-11). We divided the diversity spectrum into five classes. There is of course a continuum between low and high diversity. The dividing lines are there to guide the visual interpretation. The lines follow the most common path of the diversity curves in that part of the spectrum. Thus, the diversity of samples of different sizes may be directly compared.

By using this diversity classification system, we obtained an easy visual interpretation of the results from our studies in the fjords. Figures 8-11 show some examples. The position of the stations is shown to the left in the figure. The station shading symbols correspond to the diversity class revealed by the sample (see the legend to the figure). To the right in the figure the number of species (S) is plotted against the total number of individuals (N). We shall briefly describe the pollutant discharges to the fjords.

Orkdalsfjorden, Gaulosen and Stjørdalsfjorden. (Figures 4 and 8). Orkdalsfjorden is strongly polluted by heavy metals from a copper mine. Until recently the yearly discharges of metals in solution were 2979 tons of iron, 780 tons of zinc, 451 tons of copper, and 2.4 tons of cadmium. In the autumn of 1983, the discharges were considerably reduced. The sediments in the fjord are strongly polluted. The adjacent fjord, Gaulosen, has natural conditions similar to Orkdalsfjorden, but is much less polluted by metals. The fauna diversity was low in Orkdalsfjorden, but normal in Gaulosen. Stjørdalsfjorden is another branch of the same main fjord system. It is moderately polluted by organic materials and the southeastern part has received significant amounts of creosote. Only a slight decline in the diversity was found at one of the stations in Stjørdalsfjorden (B4).(Fig. 8).

Sørfjorden (Figures 5 and 9). This fjord is very heavily polluted by metals discharged from a zinc smelter since the beginning of this century. In 1972 the zinc smelter discharged a daily amount of 4.5 tons of lead, 6 tons of zinc, 0.3 tons of copper and 0.03 tons of cadmium [12]. The concentrations of heavy metals in the sediments, water and biota are very high [2, 9, 12]. An area of at least 30 km^2 showed a moderate, low, or very low fauna diversity.

Kristiansandfjorden (Figures 6 and 10). The northwestern part of this fjord is strongly polluted by discharges of heavy metals and chlorinated hydrocarbons from nickel industry. The diversity of the fauna in that area was moderate, low or very low.

Hvaler (Figures 7 and 11). Hvaler is the area outside the estuary of the largest river in Norway. The natural particle transport is great. There are various sources of pollution, the most important one being a titaniumdioxide factory discharging its effluents into the estuary. The diversity of the fauna was very low in the estuary and low at least 20 km seawards.

The correlation between fauna diversity and sediment concentration of selected pollutants

We wanted to test how the diversity of the soft-bottom fauna was influenced by the concentration of pollutants in the sediment. We plotted diversity ($E(S_n)$) against the concentration of copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), mercury (Hg), and polycyclic aromatic hydrocarbons (PAH) for a number of localities, and made regression and correlation analysis. Table 2 shows which fjords the material for this analyses was obtained from (se also Figure 1).

Table 2. Number of stations sampled.

	$E(S_n)$	Cu	Zn	Pb	Cd	Hg	PAH
Glomfjorden	5	5	5	5	5	5	-
Vefsnfjorden	3	3	3	3	3	3	-
Nordrana	9	9	9	9	-	-	-
Orkdalsfjorden - Gaulosen	7	7	7	7	7	7	-
Stjørdalsfjorden	14	1	1	1	1	1	13
Sørfjorden i Hardanger	7	7	7	7	6	5	-
Kristiansandfjorden	13	13	13	13	11	1	-
Frierfjorden	1	1	1	1	1	1	-
Hvaler - Singlefjorden	13	13	13	13	-	13	-
Total number of stations	72	59	59	59	34	36	13

All data were log-transformed prior to the correlation- and regression analyses. The linear regression equation, $y = ax + b$ was used, where $y = \log(E(S_n))$, $x = \log[\text{pollutant}]$, a and b are constants.

Fauna diversity is plotted against pollutant concentration in Figure 12-17. The regression line through the plot is shown. Below the plot, the equation for the regression line (Y), the correlation coefficient (R), the probability of the correlation beeing purely accidental (P), and the maximum deviation (SD) of the of the regression coefficient at confidence level 1-P, are given.

The correlation coefficients (R) between diversity and each metal, and between the concentrations of each metal, are shown in Table 3.

$E(S_n)$ and [Cu] showed a strong negative correlation (-0.79). Compared to the other metals, the data points were more densely gathered around the regression line, the regression line was steeper, the regression coefficient had a narrower confidence interval, and the correlation coefficient was farther from 0. A moderate negative correlation was shown for $E(S_n)$ with [Pb]. A low negative correlation was shown for $E(S_n)$ with [Zn], [Cd] and [Hg]. There was no correlation for $E(S_n)$ with [PAH] for the observed range of concentrations.

The strong negative correlation between fauna diversity and copper concentration may be explained as a cause-effect correlation, with high copper concentrations being toxic to a number of species, thus lowering the species diversity in the community. Both high copper concentrations and lowered species diversity may, however, have been caused by an unknown factor, e.g. some pollutant co-occuring with copper. The second explanation is improbable, since copper concentrations showed stronger correlation with lowered species diversity than with any of the measured pollutants (Table 3), and the correlation between lowered diversity and other metals than copper, and PAH, was moderate, low or nil.

The most probable regression coefficient for $E(S_n)$ on [Cu] was found to be -0.32 (Figure 12), which means that the number of species is roughly halved for each ten times increase in the copper concentration. On the basis of the existing data, a threshold value for copper concentrations which may cause toxic effects, cannot be fixed with certainty. It does seem, though, that concentrations elevated only a few times above the background levels in fjord sediments, which are 20-30 ppm dry weight [11] may have an effect. This means that copper pollution in Norwegian fjords has erased many species over large soft bottom areas. Bryan [1] stated

Table 3. Correlation coefficients.

	E(S)	Cu	Zn	Pb	Cd
Cu	-0,79				
Zn	-0,36	0,56			
Pb	-0,50	0,67	0,87		
Cd	-0,35	0,45	0,79	0,66	
Hg	-0,46	0,50	0,79	0,78	0,81

that an increase in the concentrations of some of the more toxic metals in sea water by a factor of 10 might be expected to have fairly obvious ecological effects. Copper is the most toxic metal to marine organisms with the exception of mercury and silver [1] .

Investigations of copper in an Alaskan fjord revealed that copper is easily remobilized in the sediments causing high concentrations of dissolved copper in the interstitial water [3].

Bryan [1] was concerned about the difficulty in detecting subtle ecological effects and gradual changes, and believed that effects resulting from metal contamination have gone unnoticed unless they have been extremely obvious. We believe that the study of soft-bottom fauna, combined with data on pollutant load, have lessened this difficulty.

Summary and conclusions

1. Studies in a number of fjords in Norway showed negative correlation between the diversity of soft-bottom fauna and pollutant load. An empirically based diversity classification system was developed.

2. There was a strong negative correlation between fauna diversity and sediment copper concentration. We explain this as a cause-effect correlation, with high copper concentrations being toxic to a number of species.

3. Negative correlation between fauna diversity and other metals than copper, and between diversity and PAH, was moderate, low or nil for the observed range of concentrations.

4. Copper concentrations elevated only a few times above the background levels in fjord sediments may have an effect. The number of species is roughly halved for each ten times increase in the copper concentration. Thus, copper pollution has erased many species in large marine bottom areas in some Norwegian fjords.

5. Further investigations on sediment pollutant load and soft-bottom fauna may enable us to make more precise estimates for the pollutants' ecotoxicity. Diversity measures may serve as criteria for the

ecological effects of pollution and indicate its area of influence. Combined with data on sediment pollutant load and discharge amounts, they may form a basis for optimizing environmental protection measures.

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Fig. 1. Map of Norway with fjords referred to in the text.

Fig. 2. The diversity curves of the fauna in samples from two fjords in Norway.

Fig. 3. The diversity curve for the whole soft-bottom cummunity in a hypothetical area. The broken lines represent a sample of common size and diversity (1000 individuals and 70 species).

Fig. 4. Diversity curves for stations in Orkdalsfjorden (B2, 5, 6, 7) and Gaulosen (8-13) (see Fig. 8).

Fig. 5. Diversity curves for stations in Sørfjorden (see Fig. 9).

Fig. 6. Diversity curves for stations in Kristiansandfjorden (see Fig. 10).

Fig. 7. Diversity curves for stations in Hvaler 1982 (see Fig. 11).

Fig. 8. Bottom-fauna stations and classification of diversity in Orkdalsfjorden, Gaulosen and Stjørdalsfjorden.

Fig. 9. Bottom-fauna stations and classification of diversity in Sørfjorden.

Fig. 10. Bottom-fauna stations and classification of diversity in Kristiansandfjorden.

Fig, 11. Bottom-fauna stations and classification of diversity in Hvaler.

Fig. 12. Correlation between fauna diversity and copper concentration.

Fig. 13. Correlation between fauna diversity and zinc concentration.

Fig. 14. Correlation between fauna diversity and lead concentration.

Fig. 15. Correlation between fauna diversity and cadmium concentration.

Fig. 16. Correlation between fauna diversity and mercury concentration.

Fig. 17. Correlation between fauna diversity and polycyclic aromatic hydrocarbon concentration.

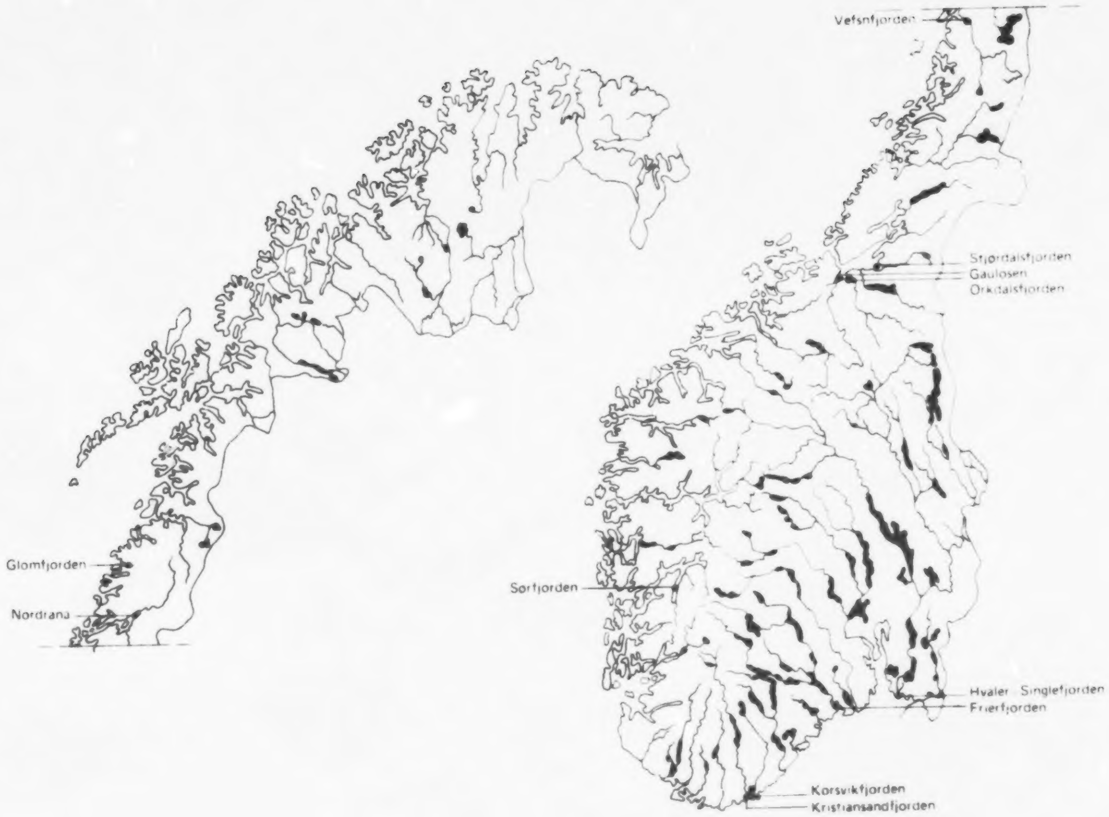

Vefsnfjorden
Stjørdalsfjorden
Gaulosen
Orkdalsfjorden
Glomfjorden
Nordrana
Sørfjorden
Hvaler Singlefjorden
Frierfjorden
Korsvikfjorden
Kristiansandfjorden

Fig. 1. Map of Norway with fjords referred to in the text.

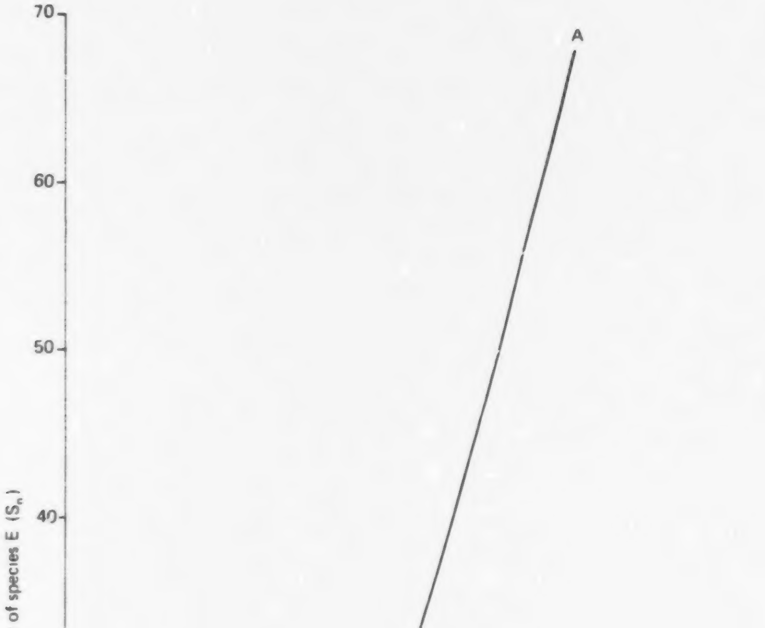
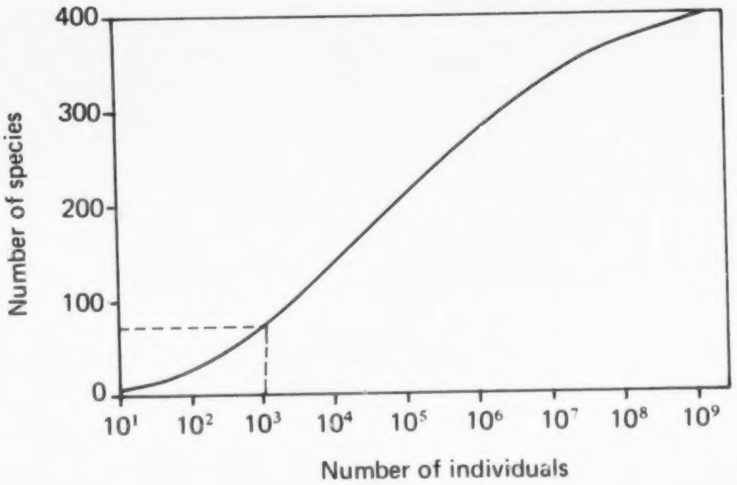


Fig. 2. The diversity curves of the fauna in samples from two fjords in Norway.



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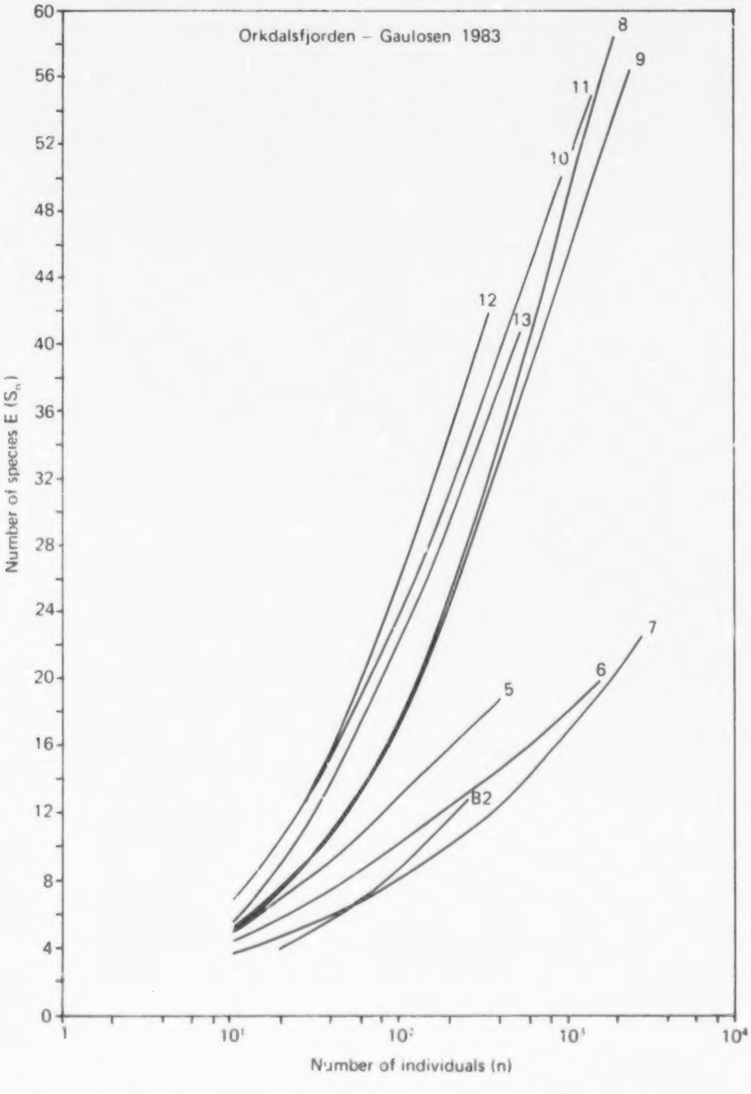


Fig. 4. Diversity curves for stations in Orkdalsfjorden (B2, 5, 6, 7) and Gaulosen (8-13) (see Fig. 8).

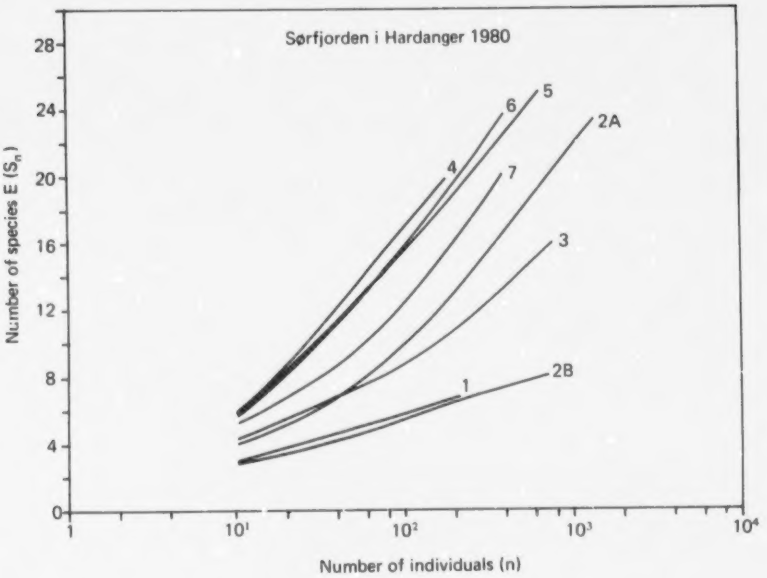


Fig. 5. Diversity curves for stations in Sørfjorden (see Fig. 9).

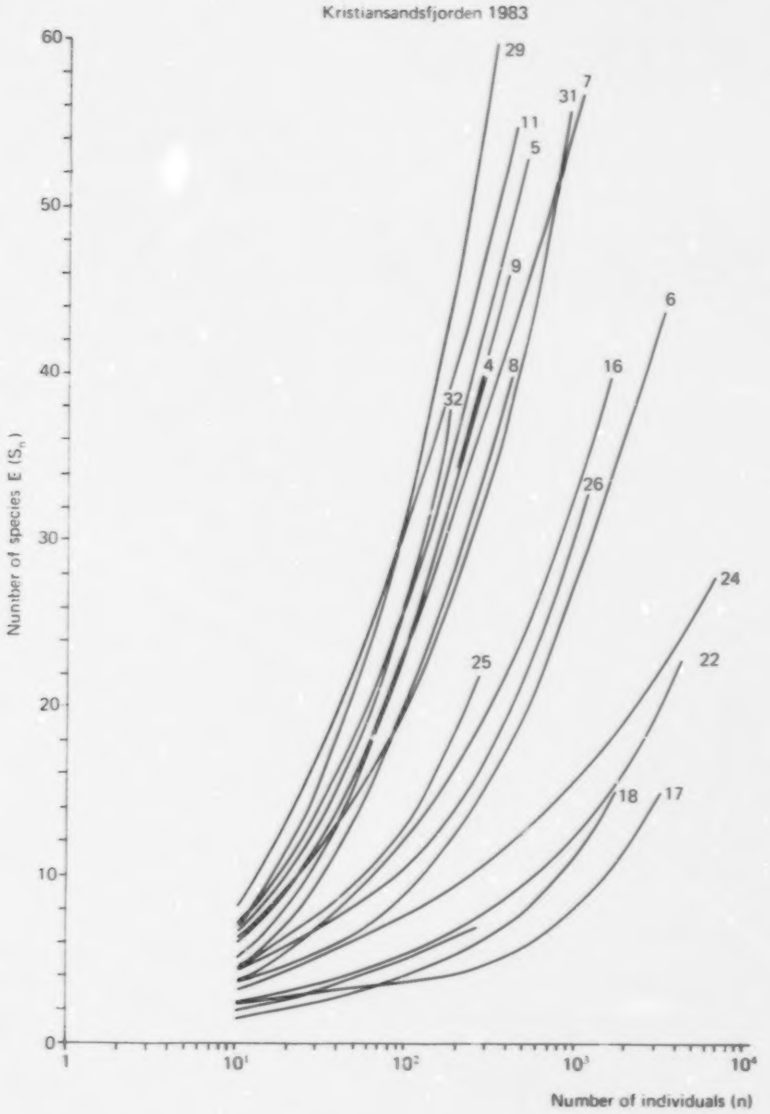


Fig. 6. Diversity curves for stations in Kristiansandfjorden (see Fig. 10).

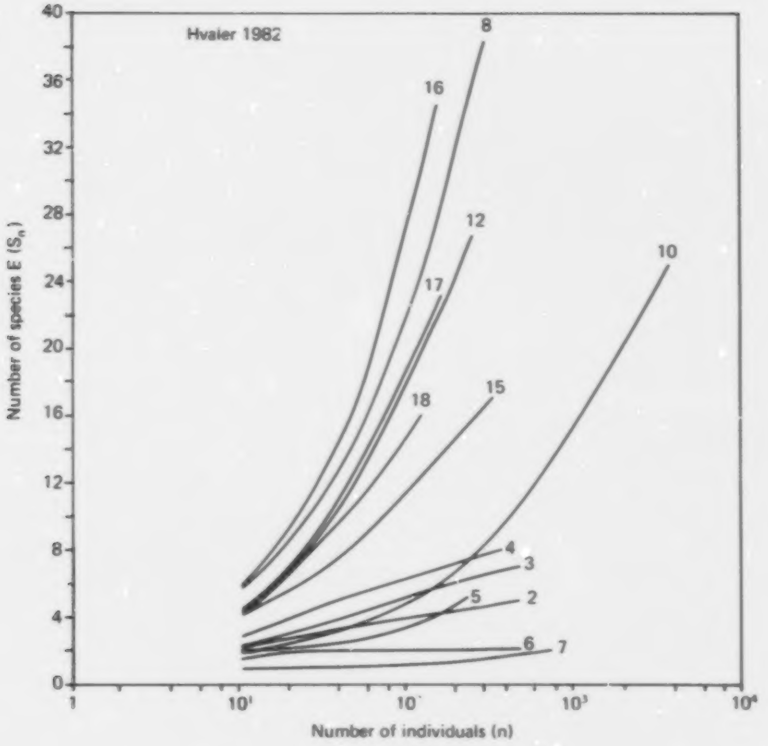


Fig. 7. Diversity curves for stations in Hvaler 1982 (see Fig. 11).

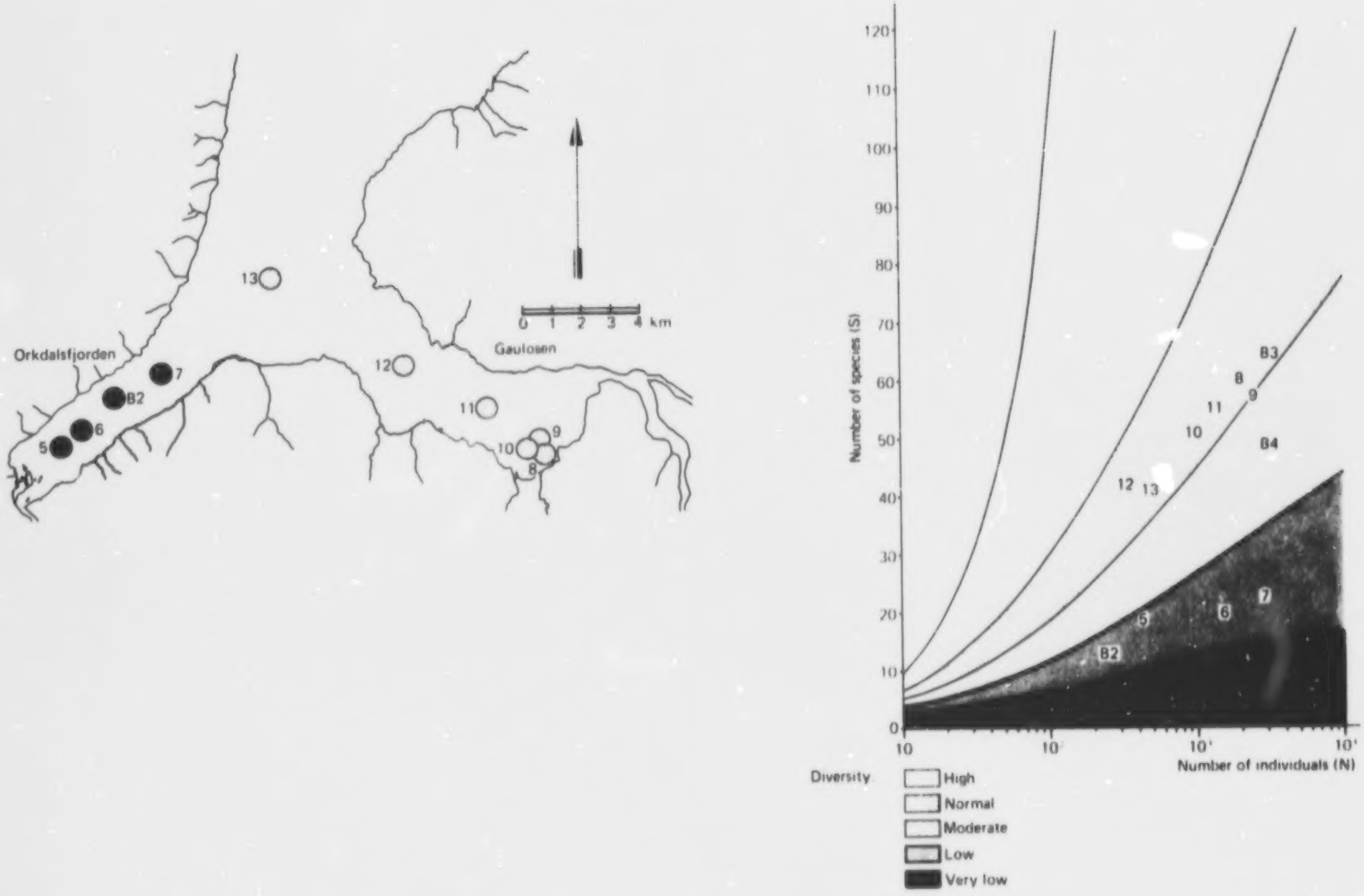


Fig. 8. Bottom-fauna stations and classification of diversity in Orkdalsfjorden, Gaulosen and Stjordalsfjorden.

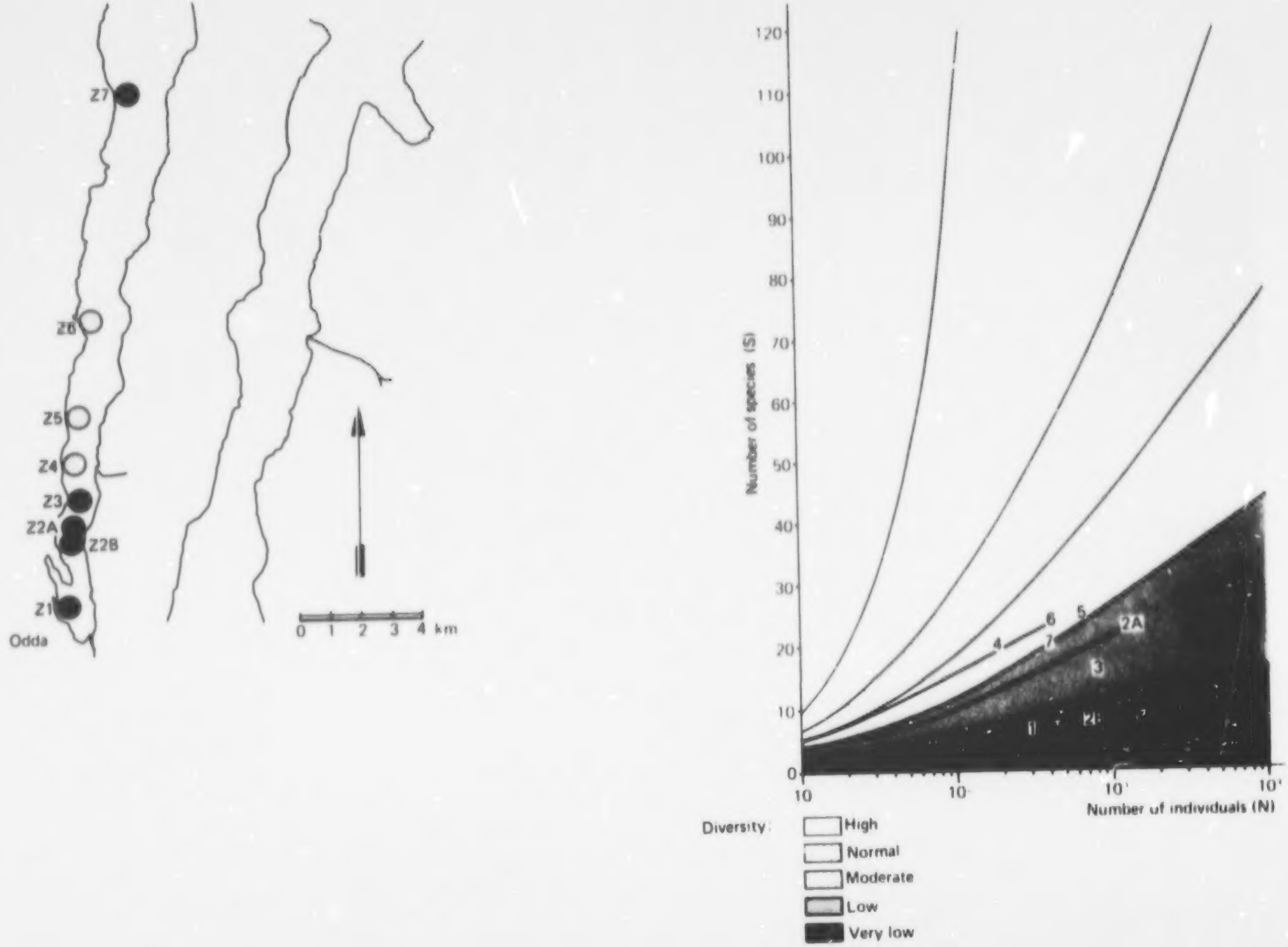


Fig. 9. Bottom-fauna stations and classification of diversity in Sørfjorden.

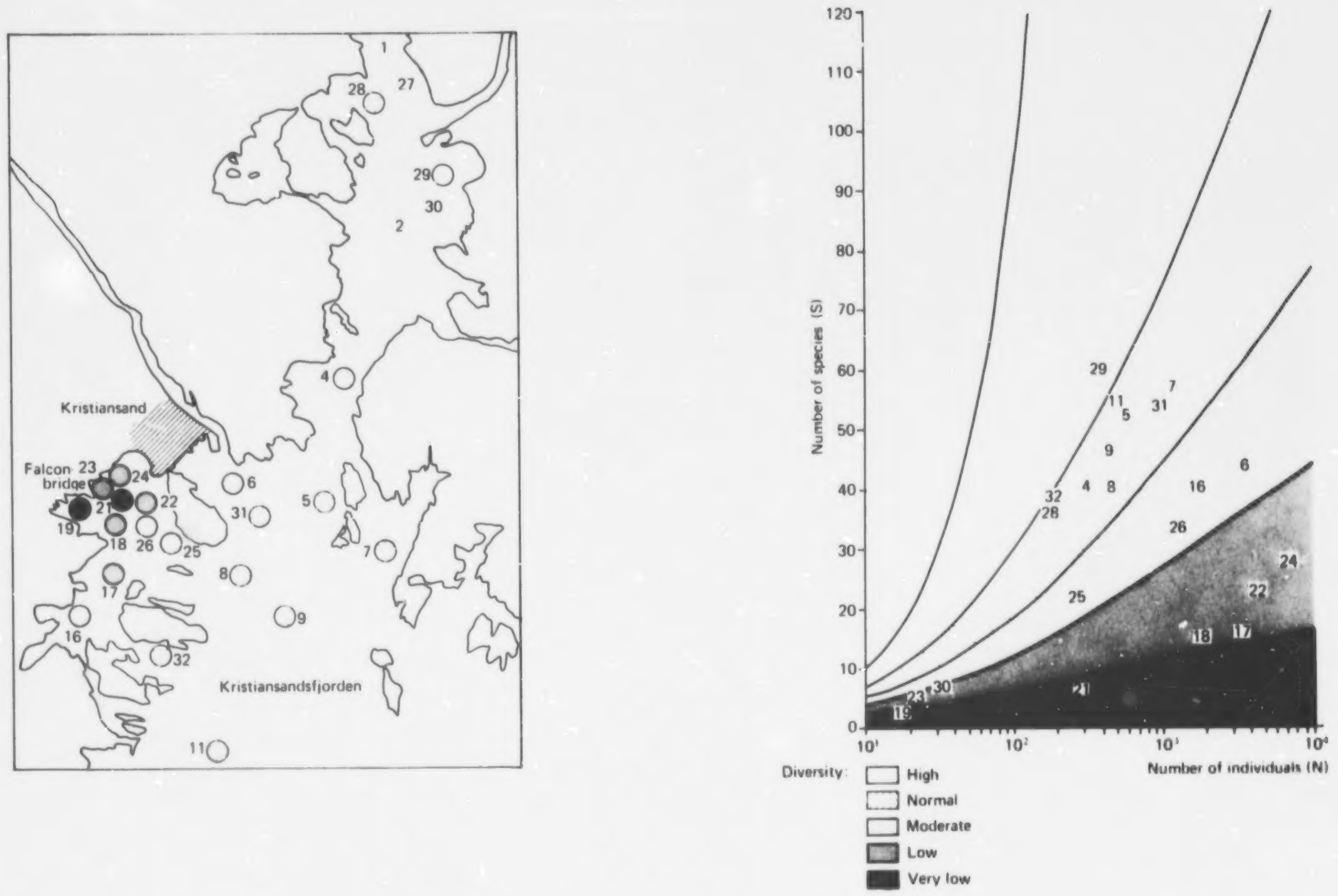


Fig. 10. Bottom-fauna stations and classification of diversity in Kristiansandfjorden.

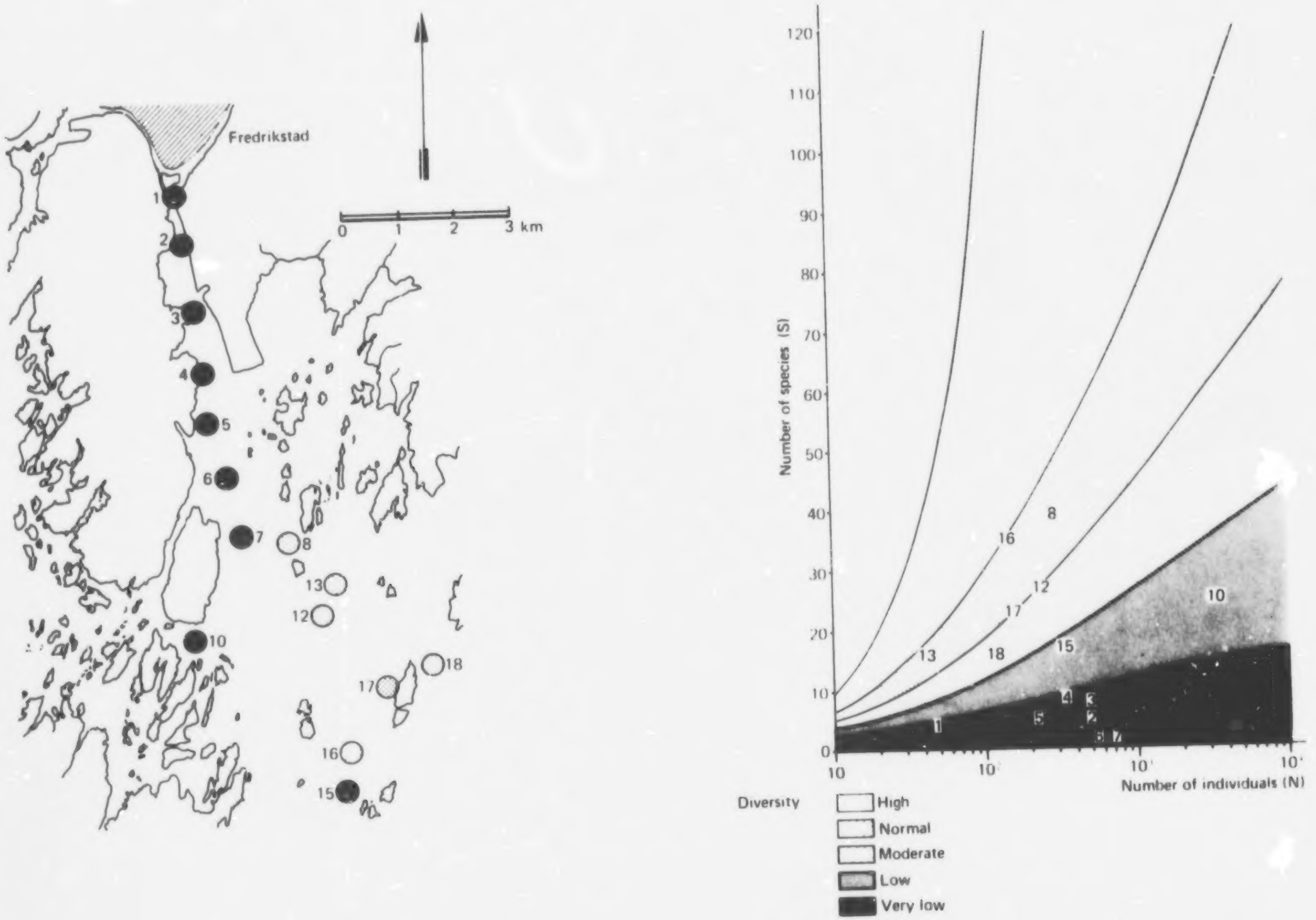
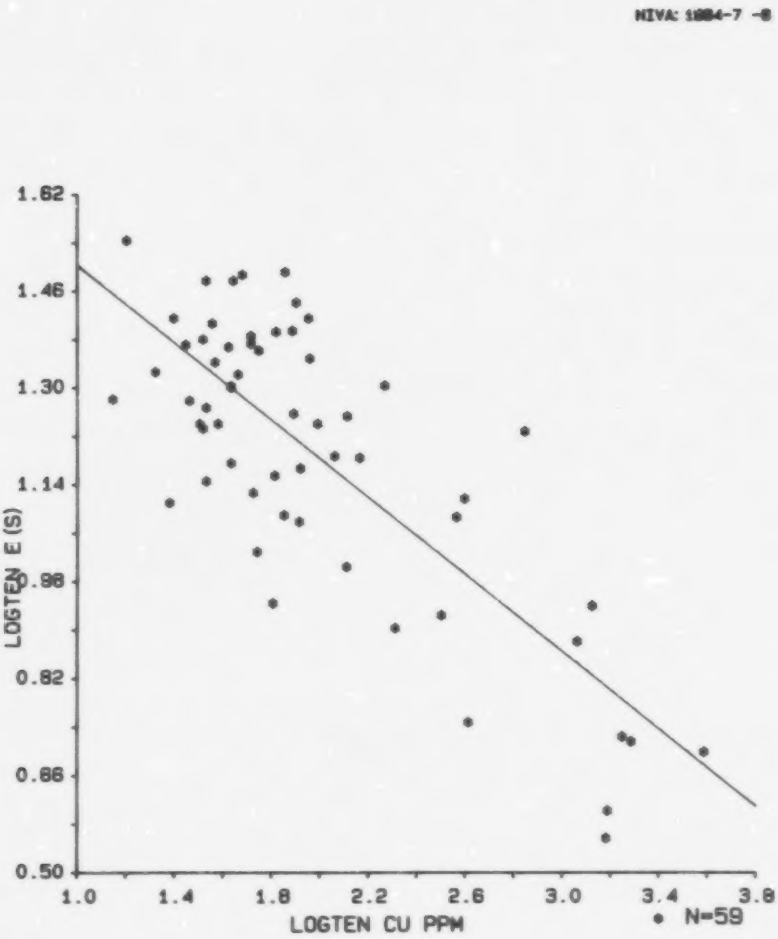


Fig. 11. Bottom-fauna stations and classification of diversity in Hvaler.



Y =-0.32X +1.82   R=-0.79   P≤0.001   SD=0.12

Fig. 12. Correlation between fauna diversity and copper concentration.

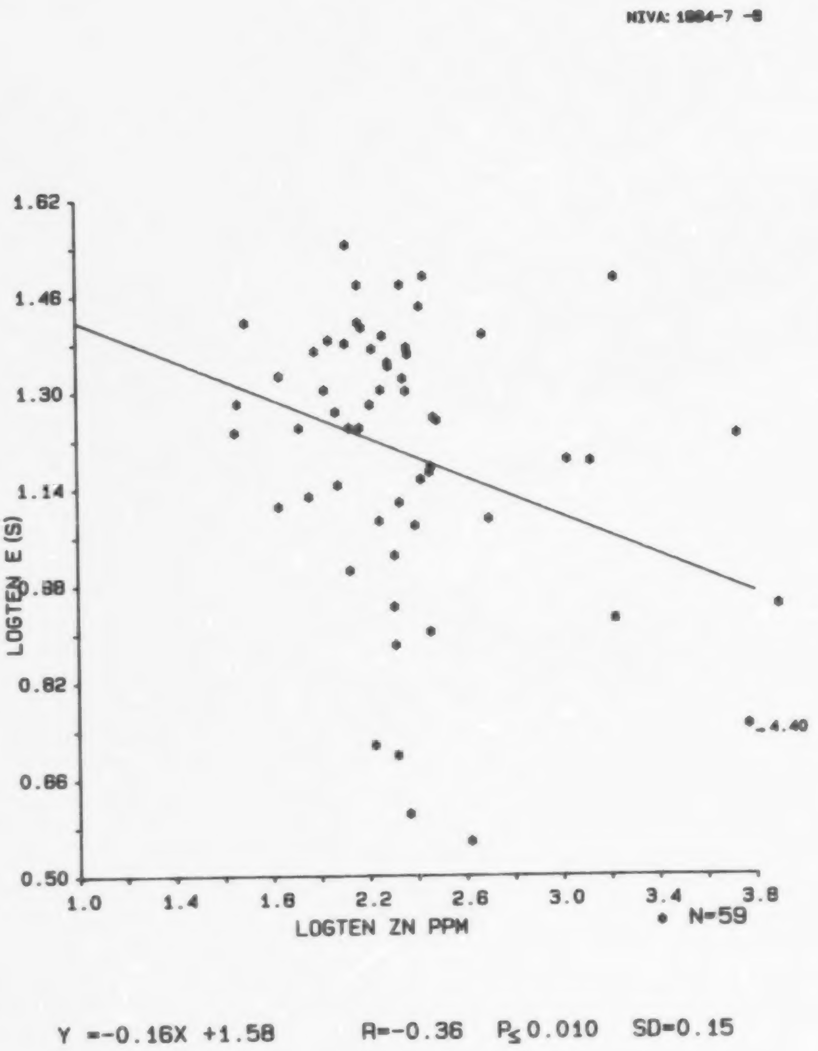


Fig. 13. Correlation between fauna diversity and zinc concentration.

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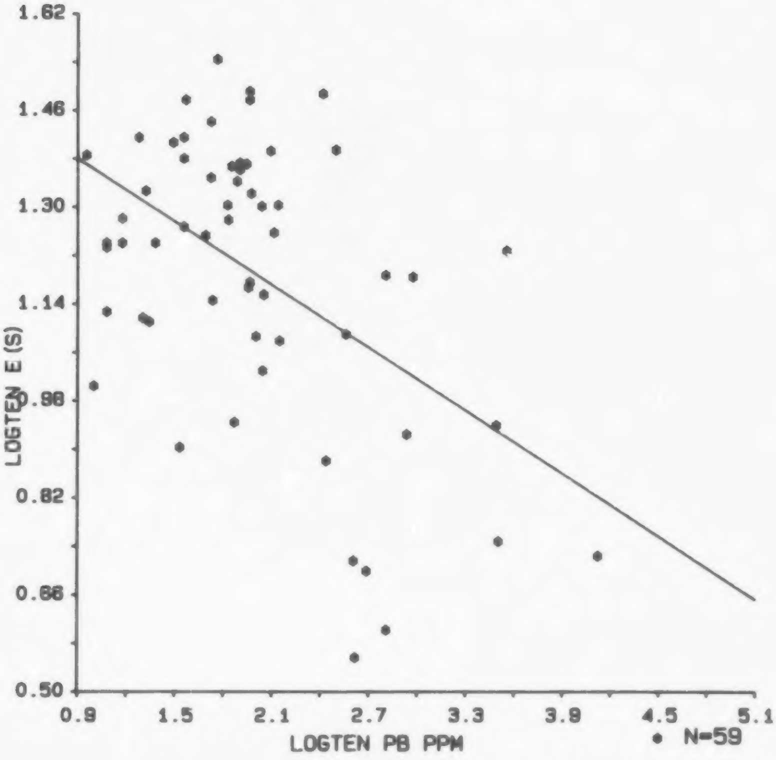
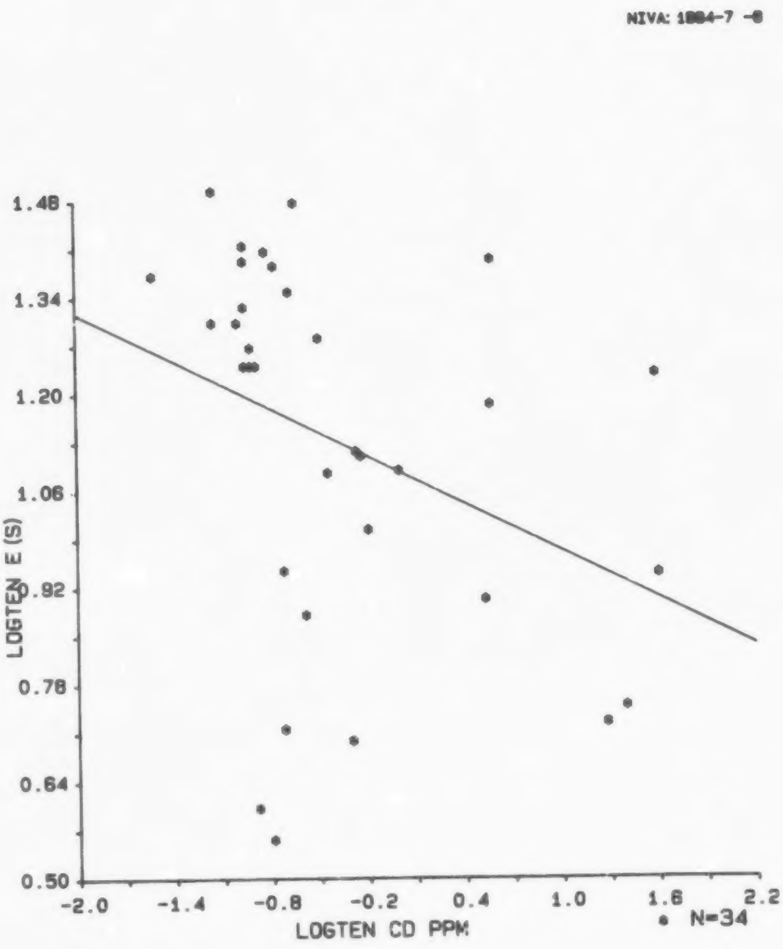


Y =-0.17X +1.54 R=-0.50 $P \leq 0.001$ SD=0.14

Fig. 14. Correlation between fauna diversity and lead concentration.



Y =-0.11X +1.09 R=-0.35 $P \leq 0.050$ SD=0.11

Fig. 15. Correlation between fauna diversity and cadmium concentration.

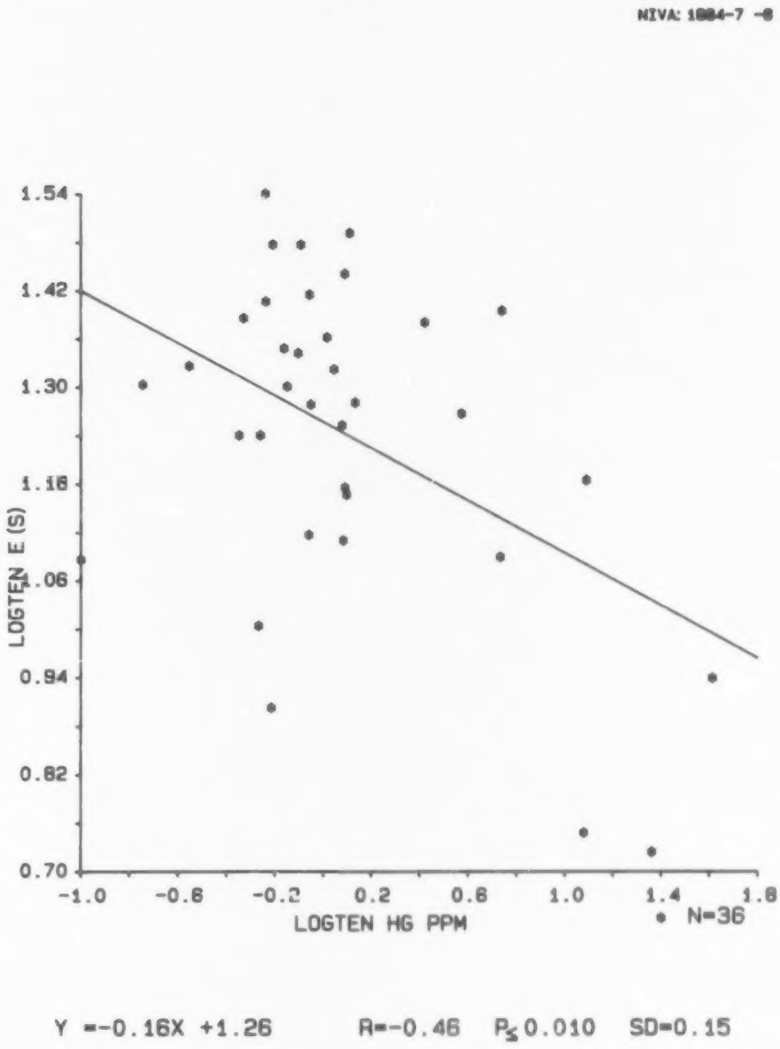
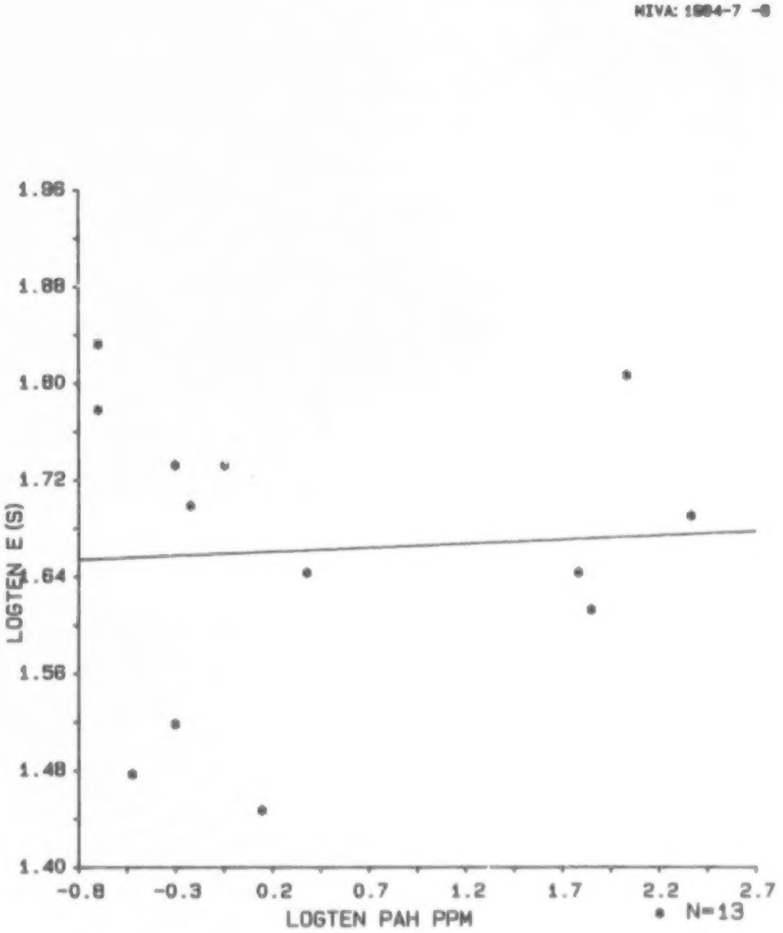


Fig. 16. Correlation between fauna diversity and mercury concentration.



Y =0.01X +1.66 R=0.06 $P \leq 1.000$ SD=0.01

Fig. 17. Correlation between fauna diversity and polycyclic aromatic hydrocarbon concentration.

APPLICATIONS OF BIOLOGICAL TESTING TO CONTROL AND LIMITATION OF WASTEWATER DISCHARGES : THE STATE OF THE ART IN FRANCE

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ABSTRACT

The authors review the different french regulations concerning water pollution control and discuss for each of them the actual use of bioassays techniques.

They state the poor development of the ecotoxicological approach in water quality management : only one standardized test with Daphnia magna is widely used for many purposes ("accidental" pollution control, pollution tax rating, screening of new chemical substances...).

However the analysis of case studies which combines toxicity testing with chemical and biological sampling demonstrates the usefulness of this approach in management strategy development for pollutant discharge limitations.

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FIGURE 2.- Schéma pour l'élaboration d'outils adaptés aux actions de lutte contre la pollution.

Le contrôle et la limitation des effets des rejets dans les eaux superficielles font l'objet de différentes dispositions de type législatif et réglementaire.

A chacune d'elles correspondent des moyens techniques variés, parmi lesquels figurent quelques rares essais biologiques, ainsi qu'il est mentionné sur le tableau 1.

Le présent exposé vise à commenter cette situation et à ébaucher des perspectives pour le développement et l'utilisation de tels essais.

TABLEAU 1.- UTILISATION ACTUELLE D'ESSAIS BIOLOGIQUES DANS LE CADRE DE LA LEGISLATION FRANCAISE

Référence légale	Loi sur la pêche	Loi sur la pollution des eaux	Loi sur la pollution des eaux + loi sur la protection de la nature + loi sur les installations classées		Loissur le contrôle et l'homologation des produits chimiques
Type d'action	REPRESSION	INCITATION	PREVENTION et CONTROLE		PREVENTION PAR PRODUITS
	Constat dans le milieu lors d'une pollution affectant la vie piscicole	Systeme mutuel de redevances et primes (Agences financières de Bassin)	Definition et revision des autorisations de rejet Par la prise en compte de normes par substances ou parametres	par etude d'impact appropriée	Examen des risques lies à la diffusion de produits nouveaux
Essais biologiques utilisés actuellement (taux d'utilisation).	Essai 24 heures sur daphnie en laboratoire (100 par an environ)	Essai 24 heures sur daphnie en laboratoire (500 par an environ)	Utilisation de normes faisant référence à des essais écotoxicologiques en laboratoire (peu sont effectués en FRANCE)	Etudes complexes de terrain et de laboratoire dans le cas des effluents thermiques. Rien pour effluents domestiques et industriels.	Essais écotoxicologiques en laboratoire (peu sont effectués en FRANCE)

I.- DESCRIPTION DE LA SITUATION ACTUELLE

1.1. Une action répressive : la polide de la pêche

I.I.I. Fondement

C'est la plus ancienne législation française visant à la protection des poissons par rapport à des déversements polluants : elle a pour origine des régles élaborées à la fin du 13ème cycle et reprises par une ordonnance de 1669. La version actuelle de ce texte fait l'objet de l'article 406 de la loi relative à la pêche et à la gestion des ressources piscicoles en eau douce votée le 29 juin 1984 :

"Quiconque a jeté, déversé ou laissé écouler dans les eaux (....) directement ou indirectement, des substances quelqonques dont l'action ou les réactions ont détruit le poisson ou nui à sa nutrition, à sa reproduction ou à sa valeur alimentaire, sera puni d'une amende de 2.000 francs à 120.000 francs et d'un emprisonnement de deux mois à deux ans ou de l'une de ces deux peines seulement (...)."

Cette législation présente plusieurs particularités qu'il convient de signaler :

- Elle est fondée sur un constat de pollution dans le milieu aquatique et ne tient pas compte des autorisations administratives données au titre d'autres législations telles que celles établissant des normes de rejet.

- Dans le cas d'effluents industriels, elle engage la responsabilité personnelle des chefs d'établissement.

- Elle permet à l'autorité administrative compétente de transiger si le responsable de la pollution prend des mesures correctives adaptées, afin de privilégier la protection du peuplement piscicole plutôt que la répression du délit.

1.1.2. Moyens techniques utilisés

Les constats sont réalisés par des agents assermentés qui disposent de plusieurs outils techniques :

- L'observation des mortalités de poissons,
- L'évaluation de la qualité biologique de l'eau à l'amont et à l'aval d'un rejet par des prélèvements de faune benthique et de calcul d'indices biotiques (15)
- Les prélèvements d'eau, de sédiments et de poissons et l'envoi à des laboratoires compétents pour la recherche de substances polluantes.

Dans la pratique, cette législation est surtout utilisée pour réprimer les pollutions "accidentelles" correspondant à des mortalités de poissons; les recherches analytiques sont principalement conduites pour détecter des substances à des concentrations létales aiguës vis à vis des poissons. Les essais biologiques réalisés en laboratoire à partir des échantillons prélevés par les agents assermentés sont donc menés à très court terme : un protocole d'essai sur daphnie (Daphnia magna S) en 24 heures a été mis au point au CEMAGREF en s'inspirant de la norme AFNOR. NFT 90301 correspondant à la ligne directrice 202 de l'OCDE (phase 1) pour détecter des différences entre les effets toxiques de plusieurs prélèvements.

Pour donner un ordre de grandeur des cas traités, les laboratoires du CEMAGREF ont été saisis en 1983 de 474 affaires de pollution dont :

19 % liées à des effluents des collectivités,
16 % liées à des effluents de l'activité agricole,
21 % liées à des effluents des industries agro alimentaires,
28 % liées à des effluents des autres industries,
16 % liées à des pollutions d'origine inconnue.

Sur ces 474 affaires, 92 ont donné lieu à des essais sur daphnies en 24 heures.

Dans 17,5% des cas traités, les résultats d'un tel essai permettent de certifier la présence de substances à des concentrations toxiques à court terme vis à vis de la faune aquatique, alors que les analyses physico chimiques donnent des résultats négatifs ou douteux, si l'on se réfère aux données bibliographiques.

Parfois, en particulier lorsqu'aucun échantillon d'eau n'a pu être prelevé à un moment opportun, des prélèvements de sédiments et de poissons peuvent être effectués aux fins d'analyse. L'interprétation de telles mesures est délicate : hormis de rares résultats concernant les teneurs de quelques ions métalliques dans les branchies de poissons (8), il n'existe pas de grille de valeurs tant pour les sédiments que pour les poissons, correspondant à des seuils de léthalité aigüe de substances toxiques vis à vis des poissons.

Toutefois, ces analyses donnent souvent des indications précieuses pour confirmer ou invalider un hypothèse émise concernant l'origine d'une mortalité de poissons.

En résumé, la police de la pêche est actuellement principalement menée par rapport à des pollutions "accidentelles"; pour son application, l'utilisation d'un essai d'immobilisation des daphnies en 24 heures réalisé au laboratoire apporte une information globale complétant utilement les analyses physico chimiques. Une telle procédure répressive constitue sans nul doute un utile "garde fou" ou "aiguillon" dans le cadre de la lutte contre la pollution des eaux, mais elle ne permet en aucun cas de conduire avec cohérence une telle action.

1.2. Une action incitative : celle des Agences Financières de Bassin.

1.2.1. Fondement

La création des Agences Financières de Bassin, prévue par la loi du 16 décembre 1964, avait pour objet de collecter les fonds nécessaires à la lutte contre la pollution des eaux, devenue indispensable en raison du développement croissant de l'urbanisation et de l'industrialisation.
Il s'agit d'un système de "mutuelle", fonctionnant selon le principe "qui pollue paie, qui épure reçoit".

1.2.2. Moyens techniques

La collecte des redevances de pollution concernant les effluents s'effectue en prenant en considération :

- Les matières en suspension contenues dans l'eau.

- Les matières oxydables, exprimées par une moyenne pondérée de la demande chimique en oxygène (DCO) et de la demande biochimique en oxygène pendant cinq jours (DBO5)

- Les sels solubles, mesurés par la conductivité de l'eau.

- Les matières inhibitrices, déterminées sur une eau décantée pendant deux heures selon la norme expérimentale AFNOR T. 90.301 : "Détermination de l'inhibition de la mobilité de Daphnia Magna Straus ".

Ces mêmes critères sont utilisés pour déterminer les quantités de pollution à éliminer par un dispositif d'épuration et servir ainsi de base à une prime versée par l'Agence de Bassin.

Le calcul des redevances et primes peut s'effectuer de façon forfaitaire, selon un barême élaboré par type d'activités ou à l'aide de mesures réelles principalement lorsqu'une contestation porte sur l'application des valeurs forfaitaires.

Cette procédure à sans nul doute contribué globalement à une diminution des rejets de matières inhibitrices vis à vis de la daphnie dans les cours d'eau : une enquête conduite auprès des principaux établissements industriels de la Région RHONE ALPES montre que les matières inhibitrices rejetées par 19 d'entre eux ont diminué de 82% entre 1973 et 1981 (7). Il faut d'ailleurs remarquer à ce propos que la CI 50 24 heures des effluents industriels à l'égard de la daphnie n'est bien corrélée avec aucun des critères classiques de pollution (DCO, DBO, conductivité, MES), ce qui montre tout l'intérêt d'utiliser un indice biologique (14) .

Toutefois, il est évident que cet outil ne suffit pas à conduire aux cohérence une action concrête de réduction des effets toxiques des rejets, un essai sur daphnie en 24 heures ne donnant qu'une indication très partielle de ces risques.

Des compléments à cette démarche font actuellement l'objet de recherches financées ou effectuées par différentes Agences Financières de Bassin.

Il faut citer tout d'abord des travaux visant à utiliser des essais de toxicité aigüe , tels que les mesures d'inhibition de la bioluminescence bactérienne de Photobacterium phosphoreum (réalisées par l'IRCHA, les Agences Financières de Bassin RHIN MEUSE et l'Institut de Recherches Hydrologiques de NANCY).

En outre, quelques essais originaux sont actuellement tentés par l'Agence de Bassin SEINE-NORMANDIE pour suivre en continu la qualité de l'eau d'une rivière : mise en place d'un test in situ sur algues (Scenedesmus subspicatus) placées en enceintes à membranes dialysantes, et d'un test de mesure de la dégradation de substances organiques (13).

D'autre part, certains travaux encouragés par les Agences concernent la recherche de compartiments des écosystèmes aquatiques adaptés aux analyses de substances micropolluantes : sédiments, poissons, mollusques, lamellibranches et briophytes ont particulièrement été étudiés à cet effet (10). L'objet de ces études est de mettre en évidence dans le milieu aquatique certaines substances dangereuses pour mieux en cerner l'origine et susciter la réduction de leur déversement.

1.3. La définition et la révision des autorisations de rejet.

1.3.1. Fondement.

Outre la création des Agences de Bassin, la loi sur la pollution des eaux de 1964 a prévu une réglementation des rejets établie en respectant l'unité de la ressource eau, c'est à dire en assurant la cohérence des actions d'amélioration de la qualité du milieu au sein d'un même bassin hydrographique en fonction des usages.

Cette procédure a été complétée ou révisée par la loi sur la Protection de la Nature (10.07.76) et la loi sur les installations classées (19.7.76) : tout rejet doit faire l'objet d'une autorisation accordée après étude d'impact et enquête publique, à l'exclusion toutefois des rejets d'importance négligeable.

Cette autorisation fixe :

- Les caractéristiques du rejet établies en tenant compte des exigences du milieu récepteur et des possibilités techniques de traitement.

- Les modalités de contrôle de la conformité de la qualité de l'effluent aux normes édictées.

1.3.2. Moyens Techniques

Cette démarche est fondée sur :

- Un constat de l'état de la rivière, la composition et l'importance des rejets qui y sont effectués

- La détermination des usages actuels de l'eau et de ceux qui sont à prévoir.

- La définition du niveau de qualité souhaitable de l'eau pour permettre l'exercice de ces usages.

- La connaissance des possibilités de traitement des différents types d'effluents.

La caractérisation de l'état des rivières et la fixation d'objectifs de qualité s'effectuent principalement selon une classification générale de la qualité des cours d'eau établie en 1971 (voir tableau 2) et comportant une correspondance avec les exigences des principaux usages de l'eau.

TABLEAU 2.- CRITÈRES D'APPRÉCIATION DE LA QUALITÉ GÉNÉRALE DE L'EAU

		S0	S1	S2	S3	S4
I	1. conductivité µS/cm à 20°C	400	750	1.500	3.000	> 3.000
	2. Dureté totale ° français	15	30	50	100	> 100
	3. Cl mg/l	100	200	400	1.000	> 1.000
	4. Capacité d'adsorption du Na	2	4	8	> 8	

		1A	1B	2	3
II	5. Température	< 20°	20 à 22°	22 à 25°	25 à 30°
III	6. O2 dissous en mg/l (2) O2 dissous en % sat.	7 > 90 %	5 à 7 70 à 90 %	3 à 5 50 à 70 %	milieu aérobie à maintenir en permanence
	7. DBO eau brute mg O2/l	< 3	3 à 5	5 à 10	10 à 25
	8. Oxydabilité mg O2/l	< 3	3 à 5	5 à 8	
	9. DCO eau brute mg O2/l	< 20	20 à 25	25 à 40	40 à 80
IV	10. NO3 mg/l			44	44 à 100
	11. NH4 mg/l	< 0,1	0,1 à 0,5	0,5 à 2	2 à 8
	12. N total mg/l(Kjeldahl)				
V	13. Saprobies	Oligosaprobe	β Mesosaprobe	α Mésosaprobe	Polysaprobe
	14. Ecart de l'indice biotique par rapport à l'incide normal (3)	1	2 ou 3	4 ou 5	6 ou 7
VI	15. Fer total mg/l précipité et en sol	< 0,5	0,5 à 1	1 à 1,5	
	16. Mn total mg/l	≤ 0,1	0,1 à 0,25	0,25 à 0,50	
	17. Matières en susp. totales mg/l (4)	≤ 30	< 30	≤ 30 (= dec ≤ 0,5 ml/l)	30 à 70 (= dec. < 1 ml/l)
VII	18. Couleur mg Pt/l	< 10 (absence de coloration visible)	10 à 20	20 à 40	40 à 80
	19. Odeur	non perceptible		ni saveur, ni odeur anormales	Pas d'odeur perceptible à distance du cours d'eau
	20. Subst. extractibles au chloroforme mg/l	< 0,2	0,2 à 0,5	0,5 à 1,0	> 1
	21. Huiles et graisses		neant	traces	présence
	22. Phénols mg/l		≤0,001	0,001 à 0,05	0,05 à 5,0
	23. Toxiques	Normes permissible pour la vocation la plus exigeante et en particulier pour préparation d'eau alimentaire		Traces inoffensives pour la survie du poisson	
	24. pH		6,5 - 8,5 6,0 - 8,5 si TH < 5°1	6,5 - 8,5 6,0 - 8,5 si TH 5°fr 6,5 - 9,0 photosynthé se active	5,5 - 9,5
VII	25. Coliformes/100 ml		< 5.000		
	26. Esch coli/100 ml		< 2.000		
	27. Strept fec/100 ml				
IX	28. Radioactivité	Catégorie I du SCPRI		Catégorie II du SCPRI	

Les critères physico-chimiques utilisés dans cette classification permettent de caractériser valablement les pollutions de type organique. En revanche, ils sont inadaptés par rapport à la surveillance des substances toxiques [9].

Le seul critère biologique auquel il est fait référence est l'indice biotique [15]. Cette méthode rend mal compte des effets des rejets de substances toxiques, car elle est principalement fondée sur une ordination des groupes d'invertébrés benthiques en fonction de leur degré d'oxyphilie.

En ce qui concerne les normes de qualité, qu'il s'agisse de celles figurant dans la classification de 1971 ou de celles mentionnées dans les directives européennes concernant la production d'eau alimentaire, la qualité des eaux de baignade et des eaux piscicoles et le déversement des substances dangereuses, elles ne sont actuellement le plus souvent utilisées que comme guides ; en particulier les directives européennes n'ont pas été transcrites dans le droit interne français, ce qui limite leur portée.

Par ailleurs les normes chimiques, provenant souvent d'essais écotoxicologiques réalisés en laboratoires sur substances pures, ne peuvent donner qu'une image très approximative des risques potentiels des rejets vis à vis des systèmes aquatiques.

Ainsi les moyens techniques mis en œuvre dans le cadre des procédures d'autorisation de rejet paraissent insuffisants pour traiter des problèmes posés par les pollutions autres que celles de type organique.

Ce point faible peut être parfois en partie "compensé", pour le contrôle des déversements, par la mise en place de dispositifs globaux de surveillance des effets toxiques des rejets. Sont utilisés à ce propos soit des tests réalisés à intervalles réguliers sur les effluents (essais sur daphnie en 24 heures, essais d'inhibition de la bioluminescence de *Photobacterium phosphoreum*), soit un dispositif de surveillance en continu mettant en évidence des anomalies de comportement chez des poissons exposés à l'effluent dilué [3] . [10] .

La mise en place de tels dispositifs n'en est toutefois qu'à un stade expérimental.

Il faut donc regretter que la procédure pourtant exigeante d'étude d'impact préalable aux autorisations n'ait pu permettre la création d'outils techniques adaptés à la prévision, au constat et à la surveillance des effets d'effluents autres que domestiques.

Sans doute cela est-il lié au fait qu'en France les études d'impact sont très rarement confiées à des instituts de recherche, et que peu d'efforts de reflexion scientifique aient été menés dans ce pays en matière de problématique des études d'impact [5] .

Un contre exemple à cette carence concerne l'étude des effets des rejets des centrales thermiques sur les biocénoses aquatiques, qui a donné lieu dès 1964 à la mise en place d'un comité scientifique pluridisciplinaire et a permis la réalisation de nombreux travaux de recherche concernant :

- la mise en évidence au laboratoire des effets de chocs thermiques sur la survie et la physiologie de nombreux organismes aquatiques,

- l'étude de l'influence de la température sur la toxicité de plusieurs substances,

- la mise au point et la calibration de techniques d'inventaires biologiques en grands cours d'eau (invertébrés benthiques, algues, poissons),

- la "modélisation" du comportement des systèmes aquatiques par rapport à un échauffement des eaux.

Les résultats des travaux ainsi effectués sont publiés dans une revue spécialisée : "les cahiers du laboratoire d'hydrobiologie de Montereau", éditée depuis fin 1974.

La réalisation d'un tel programme d'études a permis d'établir des prévisions qualitatives fiables par rapport aux effets du fonctionnement des centrales thermiques, principalement en ce qui concerne la composition des peuplements de poissons

- de mettre en place des programmes de surveillance adaptés, comportant des observations biologiques complétant les contrôles thermiques et chimiques,

- de discuter à partir de bases objectives de la norme concernant la température dans le cadre de la directive européenne portant sur la qualité des eaux aptes à la vie du poisson.

Dans ce même cadre des activités du Comité Scientifique de Montereau, ont également été réalisées des études écotoxicologiques concernant le traitement des "salisures" des réfrigérants atmosphériques : les différentes substances dont l'application était envisagée (hypochlorite de sodium, dioxyde de chlore, produits antitartrants et dispersants divers) ont donné lieu à des essais multiples sur bactéries, algues, *dreissena*, poissons, tant au laboratoire qu'*in situ*. [6], [12]. Un protocole de chloration a été élaboré, comportant des mesures de contrôle de la purge avant rejet dans le milieu récepteur.

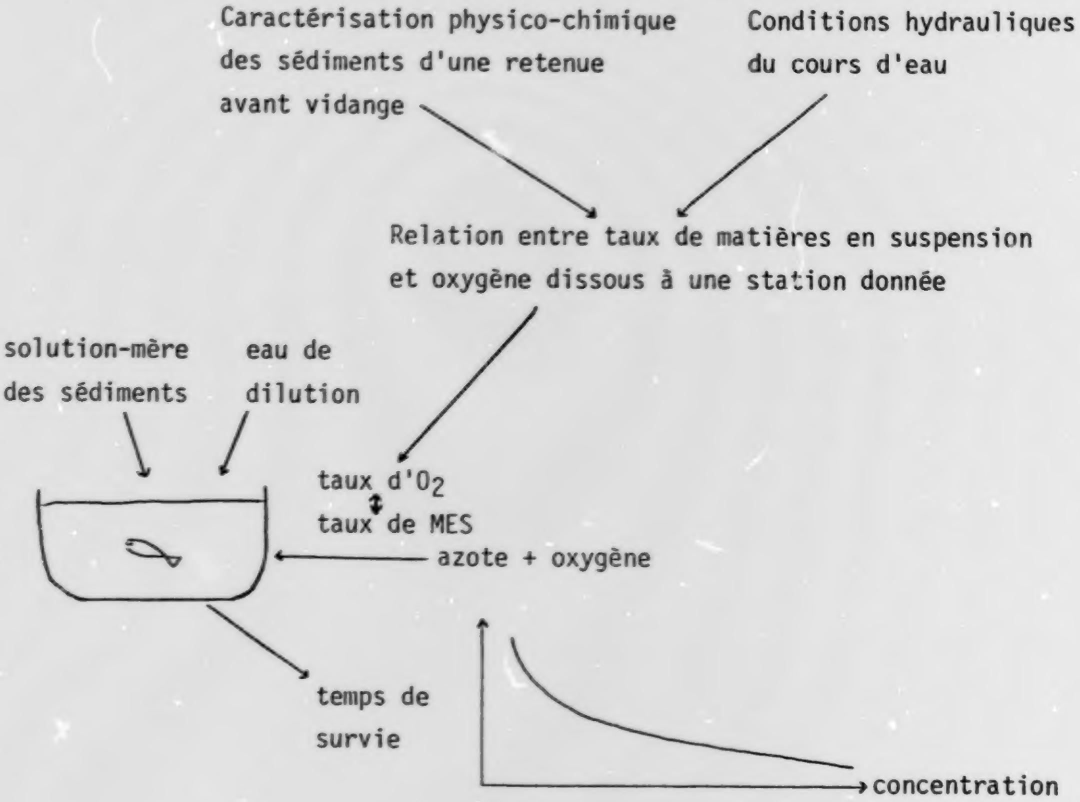
Toujours sous l'égide du Comité Scientifique de Montereau, un programme de travail est en cours pour mettre au point des méthodes écotoxicologiques permettant de prévoir les risques correspondant à la remise en suspension de sédiments occasionnée par la vidange périodique des retenues hydroélectriques (voir figure 1.)

Les premiers travaux, comportant des comparaisons entre d'une part les résultats d'essais préalables réalisés en laboratoire à partir de sédiments prélevés dans des retenues et d'autre part les observations effectuées *in situ* lors des vidanges montrent tout l'intérêt que comporte une telle démarche pour guider la conduite des opérations de vidange.

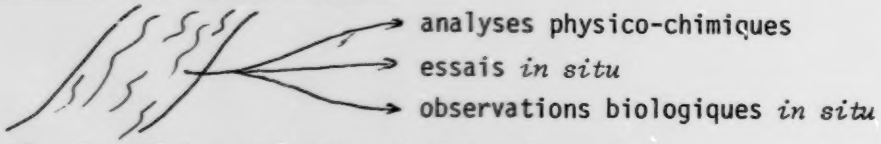
En outre, les indications données par de telles études prévisionnelles permettent d'intaller un dispositif de surveillance beaucoup plus performant.

1. Observations *in situ* des effets des vidanges : mortalités des poissons

2. Elaboration d'un essai global de laboratoire

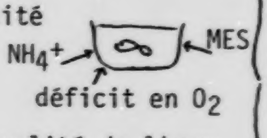

Caractérisation physico-chimique des sédiments d'une retenue avant vidange
Conditions hydrauliques du cours d'eau
Relation entre taux de matières en suspension et oxygène dissous à une station donnée
solution-mère des sédiments
eau de dilution
taux d'O_2
taux de MES
azote + oxygène
temps de survie
concentration

3. Validation des résultats sur le terrain.


analyses physico-chimiques
essais *in situ*
observations biologiques *in situ*

4. Elaboration d'un "modèle" explicatif et prédictif complet

- étude en laboratoire des effets des principaux facteurs de toxicité et de leur combinaison


NH_4^+
MES
déficit en O_2

- modèle d'évolution de la qualité de l'eau à l'aval d'un barrage (NH_4^+, O_2, MES)

estimation prévisionnelle des risques en fonction de la conduite d'une opération de vidange.

FIGURE 1 : SCHEMA DE LA DEMARCHE ENTREPRISE POUR L'ETUDE ECOTOXICOLOGIQUE DES EFFETS DES VIDANGES DE RETENUES.

Dans ces divers cas, l'apport d'essais écotoxicologiques adaptés à chaque situation a été extrêmement précieux, à condition que leurs résultats soient validés par des observations effectuées *in situ*. Cette conclusion sera reprise en compte dans les perspectives que nous ébaucherons par la suite.

1.4 Le contrôle et l'homologation des produits.

1.4.1 Fondement.

Depuis longue date, les substances pharmaceutiques, puis les produits antiparasitaires à usage agricole ont fait l'objet de procédures contraignantes d'homologation avant mise sur le marché, principalement du fait des risques qu'ils faisaient encourir à l'espèce humaine.

A partir de 1977, la législation française a prévu le contrôle, par une procédure de notification, de l'ensemble des substances chimiques nouvellement fabriquées ou importées.

Ce contrôle permet à l'administration de disposer d'informations concernant les risques écotoxicologiques liés à la mise sur le marché de nouveaux produits et de prendre toutes mesures nécessaires à la sécurité de leur emploi.

La loi a été modifiée en octobre 1982 pour tenir compte des dispositions prises au niveau des communautés européennes.

Il faut ajouter à ces dispositions générales quelques réglementations particulières, telles celle concernant l'interdiction de la mise en vente de produits détergents de biodégradabilité inférieure à 80 % et la procédure d'agrément concernant la mise en vente de produits dispersants aux fins de lutte conte les déversements d'hydrocarbures en milieu aquatique.

1.4.2 Moyens techniques.

Le "dossier de base" que doit fournir lors de la notification le fabricant ou toute autre personne qui met sur le marché une substance comprend, dans le domaine des études écotoxicologiques :

- un essai de toxicité aiguë sur les poissons,
- un essai de toxicité aiguë sur la daphnie,
- un essai de dégradation.

En fonction de la quantité de substance mise sur le marché, des essais complémentaires peuvent être demandés concernant la toxicité vis-à-vis d'une algue, les effets sublétaux sur daphnie et poisson, la capacité d'accumulation dans le poisson, voire dans une chaîne trophique ...

Peu d'essais sont actuellement réalisés en France pour l'application de cette règlementation. Ceci est sans nul doute lié à l'absence de procédure d'agrément ou d'habilitation des laboratoires.

Il vient tout dernièrement d'être remédié à cette carence par l'extension du domaine d'activités du Réseau National d'Essais, association regroupant prestateurs et utilisateurs d'essais, au contrôle des essais biologiques.

En ce qui concerne les dispersants d'hydrocarbures, des essais de laboratoire ont été mis au point pour apprécier les risques écotoxicologiques correspondant soit aux substances pures, soit à leur mélange avec des hydrocarbures [1], [4].

Enfin, pour les produits antiparasitaires à usage agricole, il faut regretter la très mauvaise prise en compte, jusqu'à une date récente, des exigences du milieu aquatique dans les dossiers d'homologation. Cet état de fait évolue actuellement de pair avec la loi sur le contrôle des produits chimiques, ce qui paraît d'autant plus nécessaire que des cultures intensives sont de plus en plus souvent pratiquées dans le lit majeur des cours d'eau.

Aussi paraîtrait-il utile, avant de donner l'autorisation définitive de vente pour un produit, de suivre des expérimentations en vraie grandeur, à l'instar de ce qui est actuellement la règle pour les substances pharmaceutiques.

D'ailleurs des observations globales et rapides (mesures de la production primaire *in situ,* tests hématologiques sur poissons stockés à l'amont et à l'aval du lieu de traitement, inventaires d'invertébrés benthiques ...) réalisées lors d'applications réelles de produits antiparasitaires sont susceptibles de fournir des indications précieuses pour l'utilisation de ces substances, ainsi que l'ont montré quelques expériences relatives à l'usage de produits débroussaillants sur les berges de cours d'eau du Sud Ouest de la France [2].

2. - ANALYSE ET PERSPECTIVES.

L'examen de la situation actuelle montre que :

- la France dispose d'un arsenal législatif et réglementaire particulièrement riche, et qu'il est donc possible d'utiliser plusieurs "leviers" pour limiter et contrôler les déversements dans les milieux aquatiques.

- les essais biologiques utilisés actuellement sont peu nombreux : seul le test normalisé de mesure de la toxicité d'un effluent ou d'une substance en 24 heures à l'égard de *Daphnia magna* est assez largement employé, et ce à plusieurs fins.

- les critères tant chimiques que biologiques utilisés actuellement sont insuffisants voire inadaptés pour donner une image fiable de l'effet global de pollutions autres qu'organiques.

- la mise en place d'études adaptées à des types d'effluents ou de pollutions s'effectue d'autant mieux qu'un programme scientifique pluridisciplinaire se met en place et que les "maîtres d'ouvrages" participent à la réflexion scientifique sous-jacente, de façon à mieux cerner l'ensemble des contraintes et possibilités techniques liées à la limitation des effets des rejets.

A partir de ce constat, il est possible de dessiner certaines perspectives dans l'intention de fournir une base de discussion aux différents intervenants, tant administratifs que scientifiques, dans ce domaine :

- PERSPECTIVES -

1. Les objectifs

Réduction des risques que présentent les effluents

- vis à vis des biocénoses aquatiques
- vis à vis de la santé humaine (alimentation en eau potable, baignade)

2. L'utilisation des moyens législatifs, réglementaires et administratifs

Afin d'éviter de multiplier les coût, et de prendre en compte au mieux l'ensemble des contraintes

ₒ privilégier
  - la participation des maîtres d'ouvrages à la reflexion concernant la politique de réduction des effets des rejets ;
  - l'autosurveillance dans le contrôle.

ₒ coordonner les procédures de contrôle : celles-ci devraient intégrer des constats *in situ* et une analyse des mesures faites par les maîtres d'ouvrages, qu'il s'agisse de police de la pêche ou de police des eaux. De même les contrôles utilisés pour les redevances pourraient être fondées sur des méthodes générales édictées pour la surveillance des effluents dans le cadre des autorisations de rejet. Un travail important d'harmonisation des procédures et de circulation de l'information serait à mettre en oeuvre.

3. Les moyens techniques.

Développer, par type d'effluent, une approche écotoxicologique complète de leurs effets biologiques, parallèlement aux études qui portent sur les effets des substances pures.

Il paraît en particulier urgent de développer de façon importante les outils techniques utilisés dans le cadre de la lutte contre la pollution des eaux. Afin d'articuler les différents types de "leviers" administratifs, il faut disposer de trois ensembles de données (voir figure 2).

1. des indices portant sur les effets globaux *in situ* de différents types d'effluents (ce sont par exemple les indices biotiques dans le cas des effluents domestiques) car

- ils correspondent exactement à un des objectifs : la protection des biocénoses aquatiques, gage de qualité générale des eaux

- ils intègrent les effets de toutes les substances polluantes et leurs interactions.

- ils permettent de prendre en compte les différences de sensibilité des grands types de milieux aquatiques.

2. des données concernant les concentrations maximales admissibles de substances toxiques car

- elles permettent une prévision des risques pour un nouveau type d'effluent.

- elles peuvent prendre en compte les problèmes d'alimentation humaine en eau, qui correspondent parfois à des normes plus sévères que celles nécessaires à la stricte protection des biocénoses aquatiques.

- elles permettent d'orienter les actions de réduction des effets d'un effluent complexe en examinant en priorité les substances à effet toxique majeur.

3. de résultats d'études toxicologiques globales des effluents au laboratoire et *in situ*, orientées en tenant compte à la fois des effets biologiques constatés *in situ* (choix des "maillons" les plus sensibles) et de la connaissance de "l'argumentaire" toxicologique des substances composant ou supposées (dans le cas des métabolites) composer l'effluent, afin de

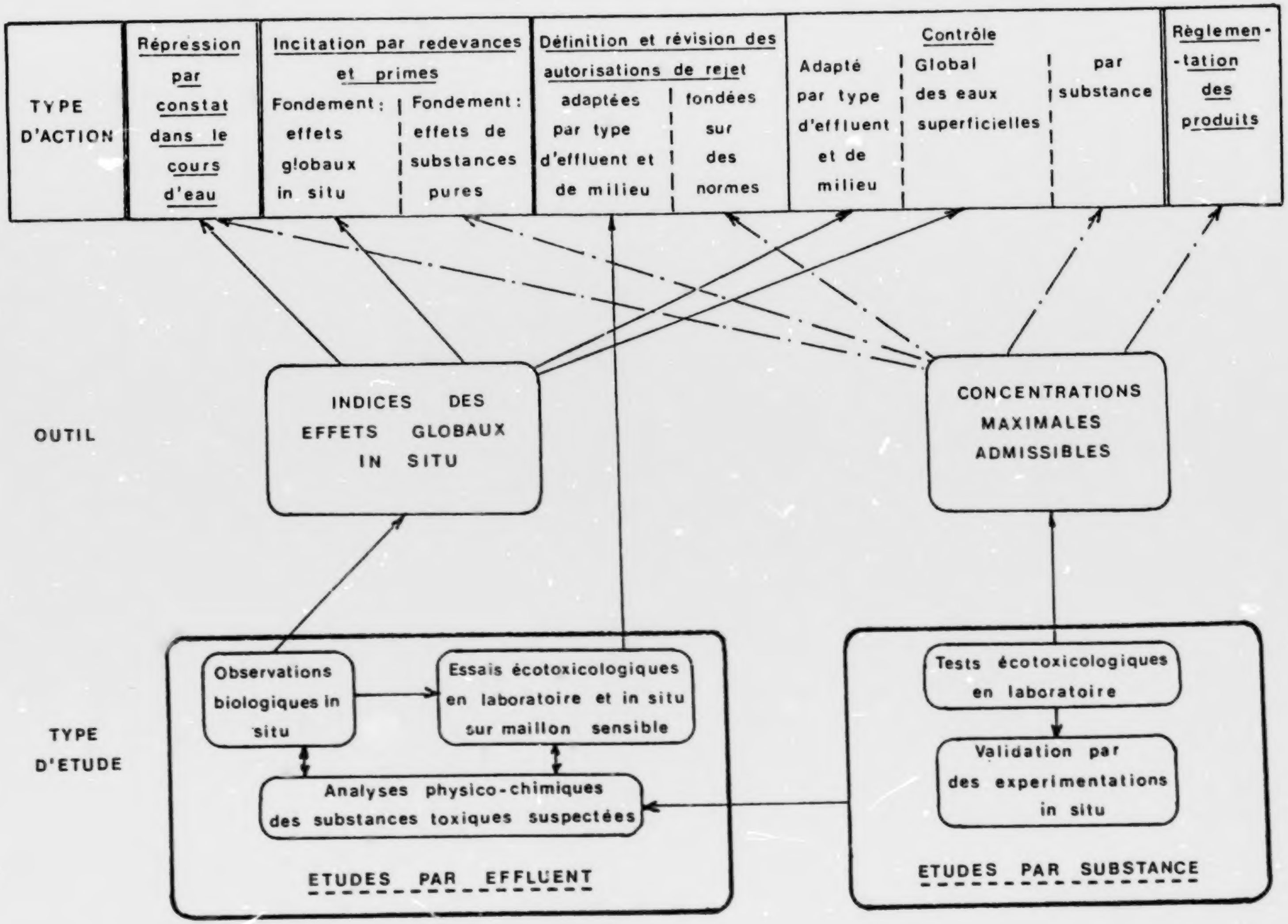

TYPE D'ACTION
Répression par constat dans le cours d'eau
Incitation par redevances et primes
Fondement : effets globaux in situ
Fondement : effets de substances pures
Définition et révision des autorisations de rejet
adaptées par type d'effluent et de milieu
fondées sur des normes
Contrôle
Adapté par type d'effluent et de milieu
Global des eaux superficielles
par substance
Règlemen-tation des produits
OUTIL
INDICES DES EFFETS GLOBAUX IN SITU
CONCENTRATIONS MAXIMALES ADMISSIBLES
TYPE D'ETUDE
Observations biologiques in situ
Essais écotoxicologiques en laboratoire et in situ sur maillon sensible
Analyses physico-chimiques des substances toxiques suspectées
ETUDES PAR EFFLUENT
Tests écotoxicologiques en laboratoire
Validation par des experimentations in situ
ETUDES PAR SUBSTANCE

FIGURE 2 : SCHEMA POUR L'ELABORATION D'OUTILS ADAPTES AUX ACTIONS DE LUTTE CONTRE LA POLLUTION.

mieux cerner les principaux facteurs (ou leur combinaison) agressant le milieu récepteur et de préconiser les techniques les plus appropriées pour la réduction de l'impact du rejet.

- - - - -

Les instituts de recherche français capables de mener à bien de telles études, en collaboration avec les laboratoires industriels privés, ne sont pas rares. Citons parmi d'autres, pour le milieu dulçaquicole, les Universités de Metz, de Toulouse, celle de Lyon en relation avec le CEMAGREF et l'Ecole Nationale Vétérinaire, l'Institut de Recherche de Chimie Appliquée, l'Institut de Recherches Hydrologiques de Nancy ... L'expérience acquise dans les pays étrangers, tant en ce qui concerne la mise au point de méthodes que leur utilisation au niveau administratif et la reflexion menée par l'OCDE à ce propos devraient permettre de réaliser rapidement des travaux efficaces dans ce domaine.

REMARQUE : Le présent document n'engage que la responsabilité de ses auteurs. Préalablement à sa rédaction, une enquête a été effectuée auprès de nombreux organismes, services et laboratoires pour connaître l'état de leurs recherches dans le domaine concerné.

Des réponses ont été aimablement fournies par :

- les Agences financières de Bassin

ARTOIS-PICARDIE : 764, Boulevard Lahure
B.P. 818
59508 DOUAI Cedex

RHIN-MEUSE : "Le Longeau" ROZERIEULLES
B.P. n° 36
57160 MOULINS-les-METZ

RHONE-MEDITERRANEE-CORSE : 31, rue Jules Guesde
69310 PIERRE-BENITE

SEINE-NORMANDIE : 10 - 12, rue du Capitaine Ménard
75732 PARIS Cedex 15

- le Centre de Recherches et d'Essais Appliqués aux Techniques de l'Eau :
5 - 7 - 9, Boulevard Louis Seguin
92700 COLOMBES

- le Centre de Documentation, de Recherches et d'Expérimentations sur les pollutions accidentelles des eaux (CEDRE) :
COB PLOUZANE
B.P. 308
F 29274 BREST Cedex

- l'Institut Français du Pétrole (I.F.P.)
14, Avenue de Bois préau
B.P. 311
92506 RUEIL MALMAISON Cedex

- l'Institut Scientifique et Technique des Pêches Maritimes (ISTPM)
  rue de l'Ile Dieu
  B.P. 1049
  44037 NANTES Cdex

- U.E.R. d'Ecologie
  Université de Metz
  1, rue des Récollets
  57000 METZ

- RHONE-POULENC Recherces
  C.R.D. - B.P. n° 5
  69151 DECINES-CHARPIEU Cedex

Ces réponses peuvent être consultées auprès des auteurs de ce document.
En ce qui concerne le milieu marin, le faible nombre de réponses à l'enquête n'a pas permis de dresser un état de la situation. Le courrier très précis de l'I.S.T.P.M. montre néanmoins qu'en matière d'effet des polluants sur les ressources biologiques, de nombreux essais de laboratoire couplés avec des expériences in situ ont été élaborés en particulier pour évaluer l'impact d'élévations de la température provoquées par les rejets de centrales électronucléaires sur le phytoplancton, les larves et adultes de crustacés. De même, pour l'étude des effets d'un choc acide sur les biocénoses phytoplanctoniques d'un milieu estuairien, une méthode originale utilisant des enceintes dérivantes a été mise au point.

Enfin, un modèle expérimental a été élaboré à partir d'observations en milieu naturel et en laboratoire pour rechercher les anomalies de calcification des huîtres creuses dans les zones fréquentées par la navigation.

- - - - - - -

AN INTEGRATED APPROACH TO ASSESS ECOTOXICITY

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ABSTRACT

The relentless growth of technology and its impact on the environment require a fundamental change in the technical aspects of our approach to assessing the hazards of toxic substances. The insidious spiral of ecotoxicity, that undesirable byproduct of human progress, will continue unabated if steps are not taken to stop it. More than ever before, the combination of biology and chemistry in ecotoxicology is proving to be an indispensable tool in efforts to reveal the effects and identify the causes of pollution. Supported by increasing international scientific awareness of ecotoxicological problems, the Environmental Protection Service of Environment Canada, Quebec Region, is promoting the application of integrated ecotoxicology to combat water, soil and air pollution. The objectives, means and usefulness of such an approach will be examined in this paper.

1. Toxic contamination and growing scientific awareness of ecotoxicity

One of the fundamental principles of the universe is duality. Just as every action produces a reaction, and evil seemingly must accompany good, the advent of technological progress in the late 19th century brought with it not only the advantages, but also all the disadvantages of science. The production and use of energy sources and chemical products and the expansion of agriculture, intended to improve our lives, have proved to be the principal causes of different kinds of pollution in recent decades. We have just begun to recognize the threats to the world around us. A number of tragedies resulting from environmental toxicity have heightened public awareness of this type of danger. Minamata (mercury), Seveso (dioxin) and Love Canal (chemical contamination) have, for example, become infamous household words. We have seen the damage wrought by certain chemical products on vegetation, animals and man, and how the release of chemical substances into water, soil and atmosphere through wastewater, incinerators and other sources often disrupts the receiving environment and harms its biotic components. The massive and insidious infiltration of alterants into the environment is called ecotoxicity. It comprises all types of collective environmental contamination, and is attributable to many factors. The effects of ecotoxicity are varied, and it often strikes unpredictably, so that it is difficult to precisely assess its overall consequences. This phenomenon is due partly to scientific ignorance in the past, and partly to man's carelessness, as he concentrates on the actions produced by his efforts while neglecting to consider the reactions. It was only in the early 60s that a new branch of the biological sciences, toxicology, began sounding the alarm. A recent outgrowth of that discipline, ecotoxicology, studies the impact of toxic substances on the environment in an attempt to identify the many causes and effects of ecotoxicity.

2. The need for an ecotoxicological approach

It is no longer possible to deny the usefulness of biological organisms in determining the extent of environmental contamination. Ecotoxicology is primarily based on the fact that living organisms constitute essential tools in the evaluation of environmental quality, since they are the ones exposed to the combined effects of ecotoxicity. This makes them the best indicators of potential or actual disruptions of ecosystems. The fate of toxic molecules released into the environment is highly complex and can be unpredictable. Hence, it is no longer possible for us to rely solely on chemical analysis to ensure environmental protection. On the contrary, an alliance of the biological and chemical sciences is more essential than ever in efforts to reveal the disruptive effects of ecotoxicity and identify its causes. Moreover, our ability to identify ecotoxic problems depends on the chemical nature of the pollutants, the biological indicators used for assessment, and the type and duration of the biological tests employed (Table 1). The final ecotoxic potential of the substance or waste under study will be determined on the basis of all the facts at our disposal, which is why an ecotoxicological approach is of capital importance.

A number of environmental strategies, which make use of ecotoxicology through field and laboratory tests, are currently under consideration as means of evaluating the dangers and risks of ecotoxicity. Laboratory tests can concentrate on one organism at a time, but if necessary can be conducted at different trophic levels. Multitrophic biological tests are also possible, as are tests that simulate micro-ecosystems. Field and laboratory biological tests have advantages and drawbacks, but both have a place in ecotoxicology. Rational use of both kinds of tests enables researchers to determine, with relative certainty, the ecotoxicity of the substance or waste product under study and the corresponding danger to biocenoces. The value of conducting simultaneous field and laboratory biological tests has gained international recognition, as was confirmed during recent international symposia on ecotoxicology (Leclerc and Dive, 1982; Persoone, 1984). The holding of this workshop is a further indication emphasizing the need for a comprehensive analytical scheme for evaluating the hazards of industrial discharges into the aquatic environment. We too are of the opinion that the biological monitoring of wastewater in order to identify ecotoxic effects is a more effective method than conventional chemical

Table 1. FACTORS RELATED TO THE ECOTOXICITY OF A SUBSTANCE

1. SUBSTANCE

PHYSICOCHEMICAL PROPERTIES	DEGRADATION POTENTIAL
- structure - volatility - liposolubility	- abiotic - biotic

2. TEST ORGANISMS

-sensitive
-representative
-simple tests

cost/effectiveness

3. EXPOSURE

- dose/concentration
- duration (short, medium, long term)
- level (acute, subacute, chronic)

INTEGRATION OF TOXIC EFFECTS

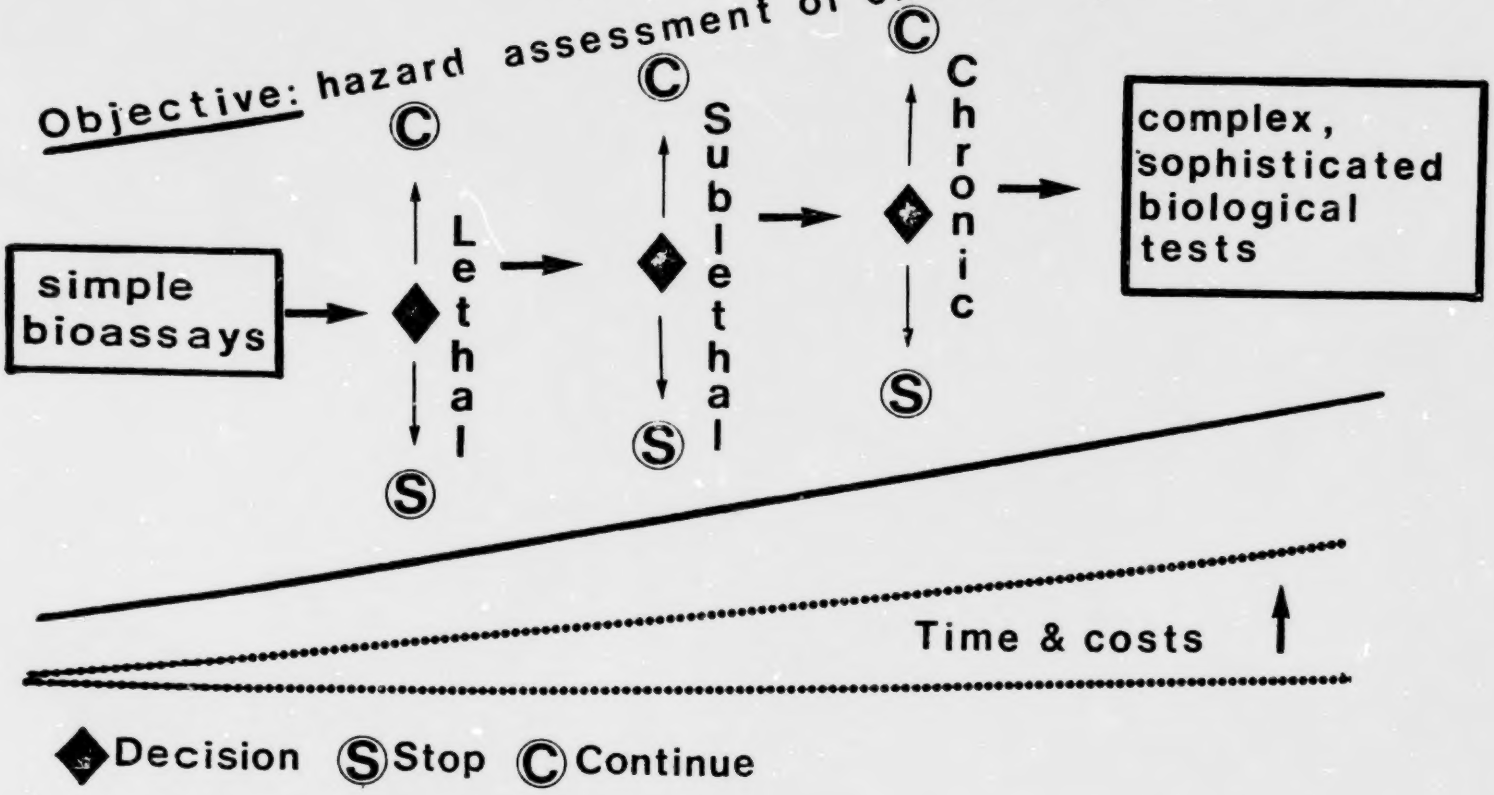
ECOTOXICOLOGICAL HAZARD ASSESSMENT

analysis alone. It is conceivable that an integrated approach for evaluating ecotoxicity of wastes released into water, which has proved both effective and economical, may later be adapted to other uses, such as the management of solid wastes and air pollution control.

The purpose of analysing a chemical product or waste is generally to determine its harmfulness and the threat it poses to the environment. The system of evaluation used will specify the lethal, sublethal or chronic effects and the bioaccumulative properties of the substance. It is impossible as yet to foresee all the short - and long-term consequences of introducing a pollutant into the environment, but an integrated approach to analysis will largely reduce the element of doubt. The schemes and procedures of current methods of global analysis of toxicity differ widely (Lloyd, 1980; Cairns, 1983). Among promising methods, one might make use of a sequential approach, or choose to conduct a variety of tests simultaneously. The "sequential" or tiered system first uses simple and inexpensive screening tests, which are followed, if necessary, by increasingly complex biological tests (Cairns, 1983). At each step, one must decide whether or not it is necessary to proceed with additional tests (Table 2). Despite the obvious value of such a method, particularly in evaluating the toxicity of pure substances, it is not the best way to analyse the ecotoxicity of wastes containing a number of substances. The sequential method has the following main drawbacks (Cairns, 1983):

TABLE 2 – SEQUENTIAL (TIERED) APPROACH.

Objective: hazard assessment of chemicals/wastes.


simple bioassays
Lethal
Sublethal
Chronic
complex, sophisticated biological tests
C
S
Time & costs
◆ Decision
S Stop
C Continue

a) It is assumed, with no scientific proof, that the biological tests performed at the beginning of the series can adequately predict the toxic effects of biological tests conducted later in the sequence with organisms representing higher levels of biological organization;

b) the initial biological tests, designed mainly to indicate lethal toxic effects, simply cannot predict the gamut of sublethal and/or chronic effects which may result from exposure to the pollutant under study;

c) because of the shortcomings noted in a) and b), the ecotoxic potential of the pollutant may be seriously underestimated;

d) the cost of the sequential process may be high in terms of time and the total cost of operations, particularly if the accuracy of the ecotoxic prediction fails to increase with the number of tests conducted;

e) the sequential method is intended mainly to identify the toxic aspect of a given occurrence of pollution. Ecotoxicity, however, also involves problems unrelated to toxicity, such as microbial contamination, enrichment, or physicochemical disruption.

The second method of integrated analysis recently considered to assess the ecotoxic potential of alterant substances is the "simultaneous" method (Table 3). It is based on the concurrent performance of a number of biological tests on organisms at various levels of biological organization, and takes account of lethal, sublethal and chronic toxicity thresholds as well as non-toxic environmental effects. It therefore indicates the main problems comprising ecotoxicity, and uses a carefully selected assortment of biological tests to provide the best assessment possible at an acceptable cost in terms of time and money. It is well structured to meet specific needs, and can be used to analyse different environmental situations, without having the weaknesses of the sequential approach. As we will see later, we have adopted a simultaneous approach for the study of environmental pollution problems.

3. The ecotoxicological concerns of the Environmental Protection Service (Environment Canada)

Pollution, as Figure 1 shows, is cumulative over time. Many of the oldest sources of pollution have not disappeared, but rather have been joined by new ones. Given the possibility of cumulative, synergistic, antagonistic, or simply unknown types of interactions among mixtures of pollutants in the environment, the threat is evidently of the utmost importance for any agency dealing

Table 3. SIMULTANEOUS APPROACH

SIMULTANEOUS UNDERTAKING OF BIOLOGICAL TESTS AT DIFFERENT

1. ECOLOGICAL LEVELS (Varied species)
2. ECOTOXIC LEVELS (Lethal, sublethal, Chronic)

OBJECTIVE →

IDENTIFICATION OF MAIN ECOTOXIC PROBLEMS

1. TOXICITY

LETHAL, SUBLETHAL, CHRONIC
CYTOTOXICITY
GENOTOXICITY

2. ENRICHMENT

EUTROPHICATION
ORGANIC LOAD

3. FATE OF POLLUTANTS

BIOACCUMULATION
BIOCONCENTRATION
BIODEGRADATION
PERSISTENCE

4. MICROBIAL PROBLEMS

FECAL CONTAMINATION
HUMAN, FISH PATHOGENS, etc
ANTIBIOTIC MULTIRESISTANCE

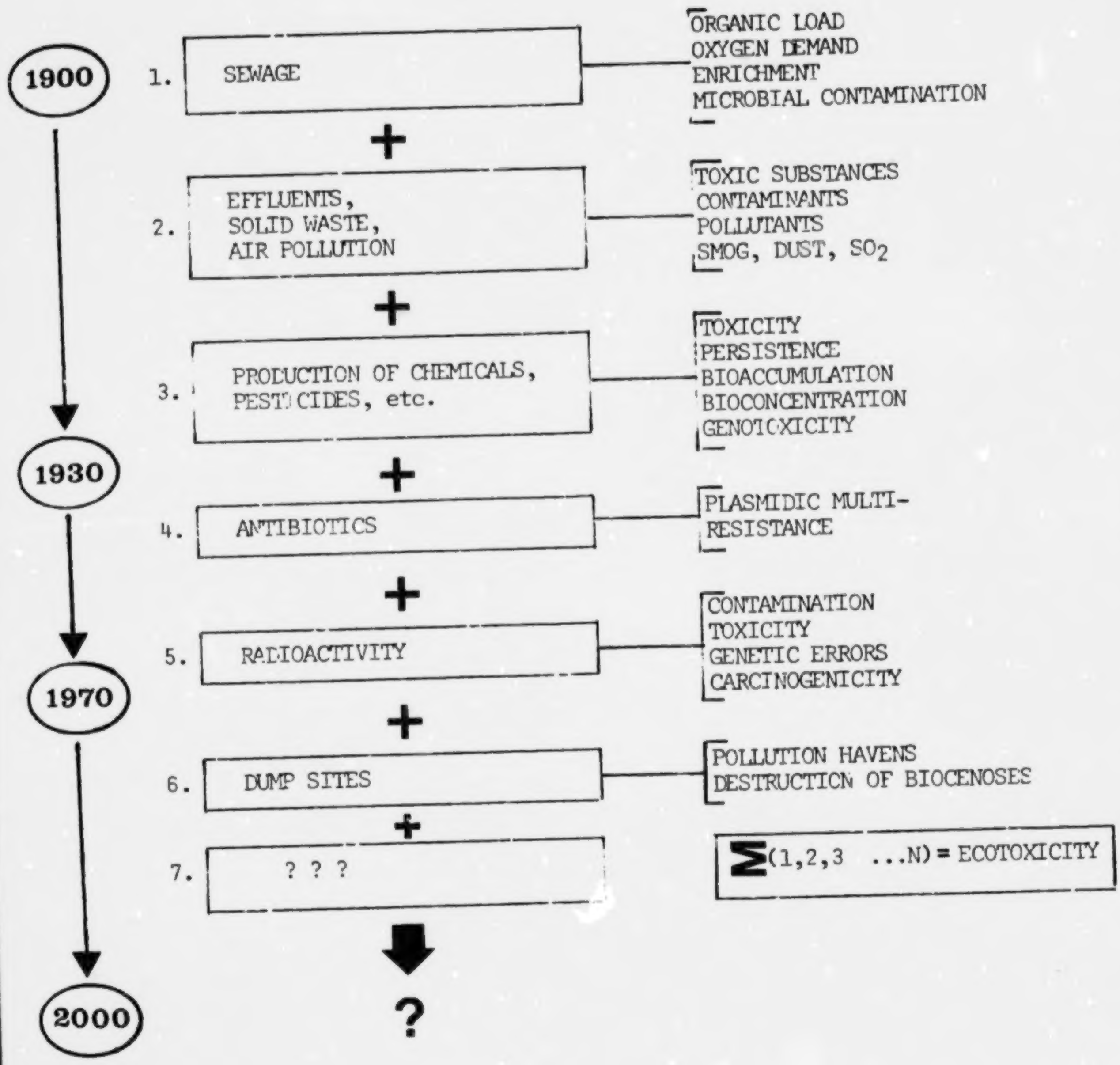

1900
1930
1970
2000
1. SEWAGE
ORGANIC LOAD
OXYGEN DEMAND
ENRICHMENT
MICROBIAL CONTAMINATION
+
2. EFFLUENTS, SOLID WASTE, AIR POLLUTION
TOXIC SUBSTANCES
CONTAMINANTS
POLLUTANTS
SMOG, DUST, SO_2
+
3. PRODUCTION OF CHEMICALS, PESTICIDES, etc.
TOXICITY
PERSISTENCE
BIOACCUMULATION
BIOCONCENTRATION
GENOTOXICITY
+
4. ANTIBIOTICS
PLASMIDIC MULTI-RESISTANCE
+
5. RADIOACTIVITY
CONTAMINATION
TOXICITY
GENETIC ERRORS
CARCINOGENICITY
+
6. DUMP SITES
POLLUTION HAVENS
DESTRUCTION OF BIOCENOSES
+
7. ? ? ?
$\sum$(1,2,3 ...N) = ECOTOXICITY
?

Figure 1 CUMULATIVE EFFECT OF DIFFERENT ENVIRONMENTAL POLLUTIONS

with environmental protection. Although the Environmental Protection Service (Environment Canada) has yet to establish a definite policy on the assessment of ecotoxicity, there is strong support within EPS in favour of such a step. An examination of recent documents on environmental problems turns up the following comments, justifying the actions taken in the Region since 1981 to implement a program for the ecotoxicological assessment of substances released into the environment. For example:

- "The Department of the Environment should take initiative in ensuring the rapid development of simple, quick and inexpensive testing procedures for determining the toxic effects of environmental contaminants..." (Chant and Hall, 1979).

- "The emission of toxic artificial chemical products into the environment is one of the greatest concerns of our time..." Environment Canada's aims are very simple. We are trying to predict problems of toxic contamination rather than engage in expensive clean-up programs later. (H&W and Environment Canada, 1983).

- "We are working in a difficult budgetary context. We have to do more. Above all, we must do better with our own resources..." (Gérin, 1983).

- "The Department feels that it is essential to develop assessment tools in keeping with the ecotoxicological approach." (Environment Canada, 1983).

- "...our activities can.be divided into three main thrusts: identification, correction, and prevention. (Slater, 1983).

In short, we see the above as pleading for efforts to develop and apply biological tests capable of producing data for ecotoxicological assessment at the lowest possible cost. Our approach was based on this requirement.

4. The mounting spiral of ecotoxicity

If no steps are taken to curb ecotoxicity, it can quickly attain alarming proportions and become almost impossible to eradicate. Figure 2 clearly illustrates its progression, in the form of a spiral made up of five different shaded bands, representing the most important trophic levels of the aquatic ecosystem (transformers, producers, primary consumers, secondary consumers and final consumers). The bands pass through four sectors defined by two perpendicular axes. The vertical axis separates lethal and sublethal effects, while the horizontal axis separates stable (acute) and chronic (dynamic) toxic effects. Sector 1 identifies a first level of environmental protection measures necessary to evaluate lethal ecotoxic

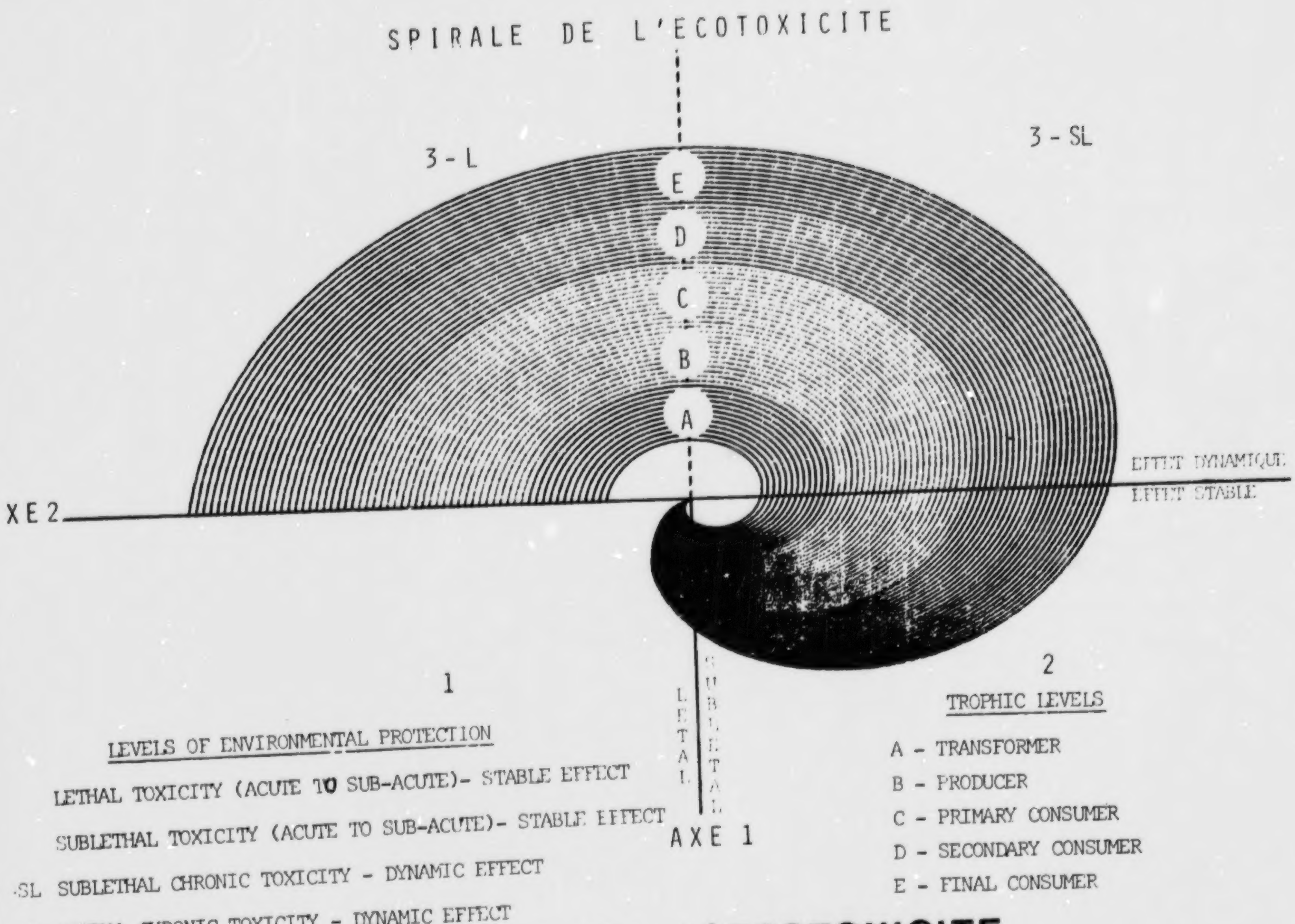

SPIRALE DE L'ECOTOXICITE
3 - L
3 - SL
E
D
C
B
A
EFFET DYNAMIQUE
EFFET STABLE
XE 2
AXE 1
LETAL
SUBLETAL
1
2
LEVELS OF ENVIRONMENTAL PROTECTION
LETHAL TOXICITY (ACUTE TO SUB-ACUTE)- STABLE EFFECT
SUBLETHAL TOXICITY (ACUTE TO SUB-ACUTE)- STABLE EFFECT
SL SUBLETHAL CHRONIC TOXICITY - DYNAMIC EFFECT
L LETHAL CHRONIC TOXICITY - DYNAMIC EFFECT
TROPHIC LEVELS
A - TRANSFORMER
B - PRODUCER
C - PRIMARY CONSUMER
D - SECONDARY CONSUMER
E - FINAL CONSUMER

FIGURE 2: SPIRALE DE L'ECOTOXICITE

effects having serious local impact. Sector 2 represents a second level of environmental protection measures which must be considered in order to assess acute sublethal ecotoxicity in the short or medium term; the impact of this ecotoxic potential is often regional. Sectors 3SL and 3L comprise the assessment of chronic toxicity (sublethal and lethal), whose impact is usually more widespread and which requires a comprehensive study before any recommendation for corrective action can be made. Chronic sublethal toxicity (sector 3SL) can gradually reach lethal toxicity levels (sector 3L) through accumulation or genotoxicity. Thus, as it extends from its starting point, the ecotoxicity spiral will first produce lethal effects, which, through dilution, will then incur sublethal effects. Then, by means of bioaccumulation, bioconcentration, persistence and genotoxicity, it will produce chronic effects in various organisms. This final type of toxicity may produce once again lethal toxicity (depending on the degree of noxiousness), to complete the toxicity cycle. A more concrete and practical representation of ecotoxicity is also given in Table 4. The following three cases of discharges into the aquatic environment will clarify the evolution of toxicity in a polluted environment.

a) Polychlorinated biphenyls (PCB)

If the initial spill has a high PCB content, it will produce lethal, sublethal and chronic effects through bioaccumulation

Table 4. OVERALL VIEW OF ECOTOXICOLOGICAL ASSESSMENT BASED ON THE THREE LEVELS OF ENVIRONMENTAL PROTECTION MEASURES

LEVEL	ECOTOXIC REPERCUSSIONS		ENVIRONMENTAL DEGRADATION		
	Level of toxicity	SCOPE	CAUSE	CONSEQUENCE	CORRECTIVE MEASURE
First	Acute lethal toxicity	Local	Flagrant toxicity: Excessive presence of pollutants (contaminants, pathogenic agents, disturbed physicochemical conditions, etc) Eg: Hg, Salmonella, thermal pollution, acid spills.	Almost complete disappearance (mortality) of biotic components of the environment, with the exception of ultra-tolerant species (eg, certain bacteria).	Primary treatment of waste.
Second	Short - and medium-term acute sublethal toxicity resulting from easily quantifiable stable effects	Regional	Presence of pollutants causing toxicity and a possible synergistic, cumulative and/or antagonistic reaction, owing to the physicochemical unbalance produced in the aquatic environment. This type of toxicity may also be biodegradable, but not bioaccumulable.	Measurable effects of disturbance on biological diversity, and physiological, biochemical and ethological stress of specific duration.	Secondary (biological) treatment of wastewater to ensure optimum harmlessness for the receiving environment must also play a role.
Third	Chronic toxicity with short -, medium - or long-term ecotoxic effects	Global	Insidious toxicity: occasionally difficult to determine its real dangers, owing to the dynamics of certain ecotoxic phenomena. Pollutants cannot be assimilated by the receiving environment and infiltrate biota through persistence, bioaccumulation and/or bioconcentration and/or by altering the genetic characteristics of the biotic components of the environment.	Reduction in the frequency of long-term survival, growth rate and reproduction of species; destabilization of structures and functions of aquatic ecosystems, with possible recurrence of lethal effects; introduction of genotoxic problems.	Elimination of this type of hazard can prove costly and often requires treatment at the source of the pollutant, following socio-economic considerations and assessment of hazards and risks.

at various levels of the aquatic ecosystem. Chronic toxicity will appear first in the form of sublethal effects, which may later become lethal.

b) Ammonia

A pollutant such as ammonia (NH_3), on the other hand, will produce no worse than acute sublethal effects, owing to biological changes which prevent it from producing chronic toxicity. It will therefore have effects limited to this level.

c) Sulphuric acid

In principle, the release of this acid should cause localized lethal toxicity (first level of toxicity), with no further consequences. In some cases, however, second- and even third-level toxic effects may result. Chronic toxicity may come about indirectly, for example, particularly in the case of continuous release of untreated acidic waste. Bioaccumulation could result from solubilization of inorganic substances in the receiving environment.

Obviously, a wide range of combinations of lethal, sublethal and chronic effects is possible, and their presence in the environment will be influenced by the type of pollutants, their migration in the environment and the likelihood of transformation.

5. The ecotoxicological approach of EPS, Quebec Region

In view of the dangers of ecotoxicity as illustrated by the spiral, an ecotoxicological approach must be tested and applied in order to identify the different aspects of this environmental scourge. Recently, the Environmental Protection Service (Environment Canada), Quebec Region, proposed the development of a practical plan for the ecotoxicological assessment of environmental hazards (Table 5). The ultimate objective of this initiative is to obtain the ability to conduct wide-range biological assessment of ecotoxic problems in the aquatic, land and atmospheric ecosystems. We feel that this ambitious program is feasible, despite financial and human resources limitations, if the ecotoxicological assessment is performed by means of simple, quick and inexpensive bioassays. Other desirable characteristics, where possible, would be miniaturization and computerization. The application of such biological tests, which must also be sensitive, representative and accurate, so that they have scientific credibility, should theoretically guarantee the success of this method of assessment. Three phases of development are planned in order to obtain the desired ecotoxicological analytical capability (Table 6). Our integrated approach to assess the aquatic environment has recently been experimented exhaustively with liquid samples from dump sites (Van Coillie et al., 1983). The results of the study confirmed the usefulness of the simultaneous ecotoxicological approach. Other similar assessments are currently being conducted on a number of industrial effluents. These preli-

Table 5. PROPOSED ECOTOXICOLOGICAL PROGRAM FOR EPS LABORATORIES, ENVIRONMENT CANADA, QUEBEC REGION, LONGUEUIL

OBJECTIVE : To provide the Region with the technical ability to identify, through the ecotoxicological approach, the main environmental problems likely to have harmful effects on water, air and soil quality.

MEANS : 1. Develop a coherent and integrated scheme of simple, rapid, sensitive, repeatable, representative and cost-effective bioassays (IDENTIFICATION OF PROBLEM).

2. Complete the biological data obtained with complementary chemical analysis (DEFINE CAUSE OF PROBLEM).

USEFULNESS: To assess hazards to man and the environment: the identification of various problems (EFFECTS) and the substances responsible (CAUSES) will make it possible to improve our knowledge and interpretation of the repercussions of spills, the release of chemical substances, and so on, and facilitate the work of managers responsible for making recommendations and supervising the application of corrective measures to repair, or at least to reduce, environmental damage.

Table 6. MAIN PHASES OF ECOTOXICOLOGICAL DEVELOPMENT

1. PHASE I : Development of a practical action plan for integrated ecotoxicological hazard assessment of the aquatic environment:

   - freshwater (in progress)
   - saltwater (in progress)

2. PHASE II : Development of a practical action plan for integrated ecotoxicological hazard assessment of the terrestrial environment (to be developed and applied).

3. PHASE III : Development of a practical action plan for integrated ecotoxicological hazard assessment of the atmospheric environment (to be developed and applied).

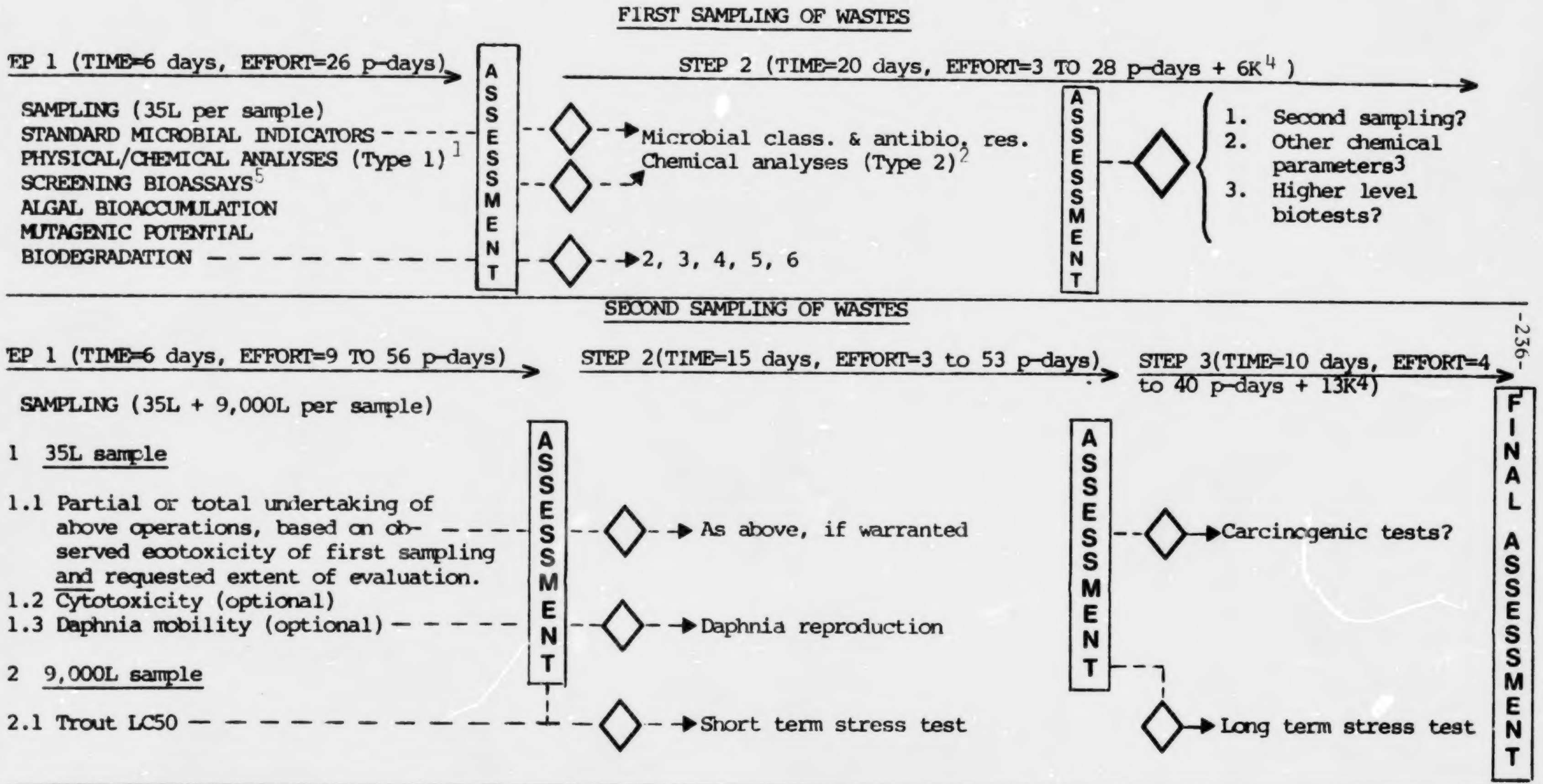
minary studies will allow us to gradually improve the experimental procedure adopted, when comprehensive interpretation of the total data collected has been completed.

6. Procedure for assessing the hazards of liquid wastes

A number of considerations must be taken into account when developing a procedure for the ecotoxicological assessment of the hazards of liquid wastes (Van Coillie et al, 1984). First, data must be obtained for the three levels of environmental protection measures corresponding to the three levels of ecotoxicity, as shown in Figure 2. There must also be a maximum time limit between sampling and subsequent analysis to ensure representativity of results. It is also essential that a number of bioassays be conducted, depending on variables such as the ecotoxicity level (ie, lethal, acute sublethal, chronic sublethal), the ecological level (from transformers up to consumers), the duration of the exposure (short-, medium- and long-term) and the choice of species and toxicity parameters. In addition, there are the specific characteristics of the samples to be analysed and problems to be foreseen in this area. Other factors to be taken into consideration are the cost of the analyses to be conducted and the number of repetitions necessary to obtain reliable results. Finally, the amount of information required by decision centres will necessarily influence the thoroughness of the ecotoxic assessment.

At present we are trying to reconcile all these considerations and have compromised somewhat, by using a method comprising different operational steps (table 7). Each step includes a number of

Table 7. SIMULTANEOUS ECOTOXICOLOGICAL EVALUATION OF LIQUID WASTES (EFFORTS AND COSTS BASED ON THE ASSESSMENT OF 10 SAMPLES).


FIRST SAMPLING OF WASTES
EP 1 (TIME=6 days, EFFORT=26 p-days)
SAMPLING (35L per sample)
STANDARD MICROBIAL INDICATORS
PHYSICAL/CHEMICAL ANALYSES (Type 1)[1]
SCREENING BIOASSAYS[5]
ALGAL BIOACCUMULATION
MUTAGENIC POTENTIAL
BIODEGRADATION
ASSESSMENT
STEP 2 (TIME=20 days, EFFORT=3 TO 28 p-days + 6K[4])
Microbial class. & antibio. res.
Chemical analyses (Type 2)[2]
2, 3, 4, 5, 6
ASSESSMENT
1. Second sampling?
2. Other chemical parameters[3]
3. Higher level biotests?
SECOND SAMPLING OF WASTES
EP 1 (TIME=6 days, EFFORT=9 TO 56 p-days)
SAMPLING (35L + 9,000L per sample)
1 35L sample
1.1 Partial or total undertaking of above operations, based on observed ecotoxicity of first sampling and requested extent of evaluation.
1.2 Cytotoxicity (optional)
1.3 Daphnia mobility (optional)
2 9,000L sample
2.1 Trout LC50
ASSESSMENT
STEP 2(TIME=15 days, EFFORT=3 to 53 p-days)
As above, if warranted
Daphnia reproduction
Short term stress test
ASSESSMENT
STEP 3(TIME=10 days, EFFORT=4 to 40 p-days + 13K4)
Carcinogenic tests?
Long term stress test
FINAL ASSESSMENT

Essential parameters to improve understanding of observed ecotoxic effects.

Parameters allowing identification of potential causes of ecotoxicity, and where analysis can be performed on easily-preservable samples.

Same remark as No. 2, but for unpreservable analytical parameters.

Funds provided for contract analyses.

Simple, rapid and inexpensive biological tests conducted with bacteria, algae and protozoa.

chemical and biological analyses to be conducted for the three levels of environmental protection measures and the three levels of ecotoxicity. Divided in this way, the operational steps do not correspond exactly to the three levels of measures or the three ecotoxicity levels considered, since each step was designed above all to facilitate the management of the process, rather than to conduct an exhaustive scientific analysis. The decision of whether to proceed from one operational step to the next will depend on factors such as:

- noxiousness observed at a given step;
- the level of assessment desired;
- the cost, in terms of the estimated effort indicated in Table 7.

In the past several months we have been evaluating this type of procedure with various effluents, adjusting it gradually and incorporating, wherever possible, miniaturized and computerized biological tests so as to further simplify operations, maximize performance and minimize costs. At the moment we are still perfecting the process. The interpretation of the mass of data generated from studies in progress will soon confirm the value of this method of ecotoxicological assessment for the aquatic environment.

As an example, we have recently applied an integrated ecotoxicological scheme to evaluate leachate samples from four landfill dump sites located in the province of Québec (Canada).

The nature of waste products contained in these sites was respectively urban-industrial, domestic, agricultural and mixed. Data tables (8 and 9) have been extracted from a follow-up report on this study (Van Coillie et al., 1984) and are presented herein. Oriented towards defining the successive local, regional and more diffuse impacts of the wastes under study, the assessment procedure employed contributed in identifying not only chemical contaminants and microorganisms of concern present in such wastes but also short - and mid-term ecotoxicity as well as potential insidious long term effects. Data integration reported in Table 8 reflects all of these ecotoxicity concerns. Among other aspects, these data clearly specify the most noxious sites in terms of overall ecotoxicity (Table 9). Of noteworthy importance is the fact that site-specific differences, in terms of varying levels and parameters of assessment, generally demonstrate corresponding tendencies of noxiousness (for example in Table 8: the highest chemical excess of 22,000 x noted for site A and the lowest chemical excess of 700x noted for site C respectively showed 50 and 0 toxic units for acute lethal toxicity). Hence, Table 9 confirms the coherence of the procedure adopted for evaluation of these wastes. In short, the overall study showed that the methodological approach used provides both diversification and maximization of information for integrated ecotoxicological characterization of dumpsite waters. The analytical process was completed within a fairly short time span in order to limit biodegradation, and the exhaustive nature of the study avoided the limitations of a tiered

Table 8. ECOTOXICOLOGICAL ASSESSMENT OF FOUR LANDFILL LEACHATE WASTE SAMPLES: DATA SUMMARY.

LEVEL OF ASSESSMENT	ECOTOXICITY	SITE A	SITE B	SITE C	SITE D
First	Chemical excess[1]	22,000X	2,100X	700X	6,100X
	Microbiological excess[1]	1,700X	40X	340X	5X
	Acute lethal toxicity (TU_L)[2]	50	4	0	12
Second	Sublethal toxicity (TU_{SL})[3]				
	a) acute (short term)	11	1	0	10
	b) subacute (medium term)	70	30	1	70
Third	Chronic toxicity (TU_{SL})[4]	12	5	2	6

1. Based on excess of standards for natural waters (Van Coillie et al., 1984): excesses reported are summations of chemical or microbiological levels analyzed which surpass a specific standard by a factor of 10.

2. Lethal toxic units (TU_L =100%/LC50) obtained with rainbow trout bioassay.

3. Average of sublethal toxic units obtained with different bioassays (P. phosphoreum, C. variabilis, S. capricornutum, D. pulex, and S. gairdneri).

4. Average of sublethal toxic units obtained following tests of biodegradation (P. phosphoreum, S. capricornutum, and S. gairdneri), bioaccumulation (S. capricornutum/P. phosphoreum), and mutagenicity (S. typhimurium).

Table 9. RELATIVE ECOTOXICITY OBSERVED IN FOUR LANDFILL LEACHATE WASTE SAMPLES.

LEVEL OF ASSESSMENT	ECOTOXICITY	DECREASING NOXIOUSNESS FOR SAMPLE SITES
First	Chemical excess	A > D > B > C
	Microbiological excess	A > C > B > D
	Acute lethal toxicity	A > D > B > C
	Flagrant ecotoxicity	A > D > B > C
Second	Sublethal toxicity	
	a) acute (short term)	A > D > B > C
	b) subacute (medium term)	A ≥ D > B > C
Third	Chronic toxicity	A > D > B > C
	Insidious ecotoxicity	A > D > B > C
	Overall ecotoxicity	A > D > B > C

approach, which, for instance, may not have revealed the mutagenic potential of site B (the only site indicating mutagenesis: Table 8, 5 TU_{sl} for chronic toxicity). Once again, this indicates the importance of conducting thorough investigations with a wide range of parameters, so as to avoid the pitfall of underestimating ecotoxicity.

7. CONCLUSIONS

The integrated method of ecotoxicological assessment is the approach best suited for studying the toxicity of water samples. A comparison of various methods showed the efficiency of a "simultaneous" approach for the analysis of industrial effluents containing numerous potential pollutants. In addition, the method is adaptable to the study of soil and air pollution. It is time for a more realistic approach to evaluate environmental pollution. Such an approach should include the analysis of a sufficiently wide range of chemical, biological and ecotoxic parameters, so as to avoid an under estimation of disturbances or toxicity. This method allows researchers to make a fairly complete assessment of alterants, since it identifies not only polluting chemical and biological substances, but also degrees of toxicity (lethal, sublethal and chronic). Moreover, an integrated method of ecotoxicological assessment is undeniably valuable in controlling pollution caused by industrial waste, because it:

a) makes it possible to improve the final quality of waste released into the environment, by showing the effects of alterant substances through biological tests and identifying clearly the industrial step or process responsible for the pollution;

b) enables us to obtain precise data on the real impact of a polluting substance on the environment, by further conducting field tests to confirm or disprove the initial concerns suggested by laboratory biological tests.

Applied for this purpose, integrated ecotoxicological assessment would encourage a preventive, rather than corrective, environmental action, and should normally help to eliminate or at least slow down a growing pollution threat which characterizes our century and, more specifically, our generation.

In short, the following points recall the necessity for conducting an adequate assessment of the complex repercussions of ecotoxicity. Such considerations, we feel, make an integrated method of ecotoxicological assessment the best way of solving them:

a) large quantities of harmful substances are released into the environment by various means;

b) this overall insult on the biosphere is called ecotoxicity;

c) environmental control agencies throughout the world are deeply concerned with the fate of chemical substances released into the environment;

d) the integrated assessment of toxic assault is done through ecotoxicology; some methods try to identify the effects and causes of ecotoxicity in order to improve environmental protection and quality;

e) an alliance of the biological and chemical sciences is essential in order to solve the problems of ecotoxicity;

f) the value of an integrated approach to ecotoxicological assessment should be recognized in environmental protection programs, and its use promoted in future;

g) environmental standards, regulations and legislation, as well as industrial technology, should be modified to take account of ecotoxicity.

. . . .

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Relationships Between the Biological Testing of Industrial Effluents and the Quality of Receiving Waters - Canadian Approaches and Examples

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ABSTRACT

A summary of selected Canadian experiences in the application of biological tests for controlling and monitoring effluent quality, for setting water quality objectives, and for monitoring receiving waters is presented. A brief review is made of the approaches for applying biological tests to effluents, to the selection of suitable water quality objectives, and to the conduct of ambient biomonitoring programs. Two case examples from Canada, the Saint John River, New Brunswick and Canagagigue Creek, Ontario, are evaluated in detail. A number of other Canadian sites where biological tests are being used are also discussed. The contributions of biological tests on effluents to administrative decisions for pollution control, as well as the limitations of solely using "end-of-pipe" tests, are emphasized in the paper.

1. INTRODUCTION

Wastewater effluents from industries and municipalities are major sources of pollutants to aquatic ecosystems. Many effluents and their receiving systems have been studied over the past few decades, and effluents in many countries are now subjected to some treatment prior to discharge. The choice of treatment depends upon several factors, including the nature and quantities of the offending substances, characteristics of the receiving water bodies, size of plant and water usage, available treatment technology, and cost of treatment.

It is widely accepted that the biological testing or biomonitoring of effluents includes "(1) the measurement of the biological properties of effluents, using captive organisms exposed to the effluent in the laboratory or in the environment, and (2) measurement of the effects of effluents on the biological integrity of the receiving waters" (42). Many of the applications of "end-of-pipe" biological tests and their limitations are well-known, as are the advantages and disadvantages of identifying and resolving effluent problems solely through field-oriented chemical and biological approaches.

The objectives of "end-of-pipe" and field approaches are to identify substances that are causing or could cause damage to aquatic ecosystems, and to monitor the efficacy of selected control measures. The aim is to detect potential problems at an early stage, to understand the environmental protection gained from treatment, and to choose effluent limits and water quality objectives that prevent costly over- or under- protection.

This paper discusses some of the applications of biological tests in the study and control of effluents and the setting of water quality objectives for major classes of pollutants. We present summaries of several Canadian studies where water quality surveys, and biological and chemical testing at source and in ambient environments, have identified serious problems and are helping to resolve them. The various contributions of biological testing to each unique study are evaluated. The essential conduct of linked studies on effluent discharges and their receiving waters is discussed.

2. THE APPLICATION OF BIOLOGICAL TESTS

2.1 Biological Testing of Effluents

Weber (42) states that "although the relative toxicity and biostimulatory properties of effluents can be measured prior to discharge, the data are of very limited use in predicting the effects of effluents on the biological integrity (standing crop, taxonomic composition, condition) of communities of indigenous aquatic organisms in receiving waters". This is a quite common commentary on "end-of-pipe" biological tests, particularly ones conducted in the laboratory using single-species tests (6). However, basic facts and knowledge can be gained from various tests directly on effluents, particularly where they are highly toxic and are being discharged into relatively small water bodies. Biological (ecotoxicological) tests have been developed and applied in Europe and North America in a sound and methodical manner, as shown below and by Mount (30) and Alabaster and Lloyd (1). As testing methods improve and the sciences of applied aquatic toxicology and environmental chemistry develop, the predictive function of many tests will improve.

Meanwhile, a number of questions about an effluent can be answered with tests directly on them (24): (1) Is the final effluent acutely toxic to aquatic organisms at the point of release? (2) How toxic? Is it toxic when highly diluted? (3) Is the toxicity variable and is such variation correlated with changes in in-plant processes? (4) What are the major substances contributing to toxicity? (5) Does treatment reduce aquatic toxicity? (6) Are the substances that are causing toxic effects likely to persist, bioaccumulate and cause toxicities, or change to other materials with similar properties, under ambient conditions? Questions 1-5 can be addressed with aquatic toxicity tests and chemical analyses applied directly on effluents. The last question often requires short and longer term laboratory and field experimentation, together with knowledge of the environmental chemistry and fate of the substances or effluent components of concern.

By the late 1960's, Canada had many industries that were releasing large volumes of effluents that either had immediate and often visible effects on biota and habitats, or largely unknown environmental consequences. Hence, for most of the 1970's, Canada's federal approach for control of effluents was (1) to control discharges by the industrial sector through regulations and guidelines, then (2) to monitor the effectiveness of controls and make adjustments, based on a plant-by-plant, site-by-site experience (35, 43). The first step included putting an aquatic toxicity protocol and specific toxicity criteria into the regulations and guidelines promulgated by Environment Canada from 1971 onwards. An acute toxicity testing protocol using rainbow trout (Salmo gairdneri) was adopted, developed and applied to effluents which could be rendered non-lethal (9, 35, 44). The objective was to improve effluent quality so that fish could survive in full-strength effluents, or allowable dilutions, for four days. The application of a relatively simple toxicity test for controlling effluent quality was the first step to preventing serious water pollution, and proved to be a very valuable screening procedure.

Work on the second step was soon begun, with studies of acute toxicity reduction in treatment systems (7, 28), and of the sublethal toxicity of treated effluents (40, 45, 46). More biological and chemical studies of selected "priority" chemicals were conducted in receiving waters near industrial outfalls and sites (see below), or near areas of large-scale chemical use (e.g. pesticides). A number of sensitive sublethal toxicity tests can be applied to effluents, some of which (such as the Daphnia reproductive test and the algal bioaccumulation tests) are considered to be sensitive and to be predictive of potential biological harm (2,24). The objective is to test treated effluents in ways that are practical, and linked to predicted exposures in ambient waters. In this way, the environmental significance of treatment can be determined, and decisions made accordingly.

Both steps (applying practical, short-term biological tests to toxic effluents, and monitoring the effectiveness of initial treatment of the problem) are continuing in Canada but at a pace slowed by both the economic recession and a reassessment of approaches to solving complex environmental problems.

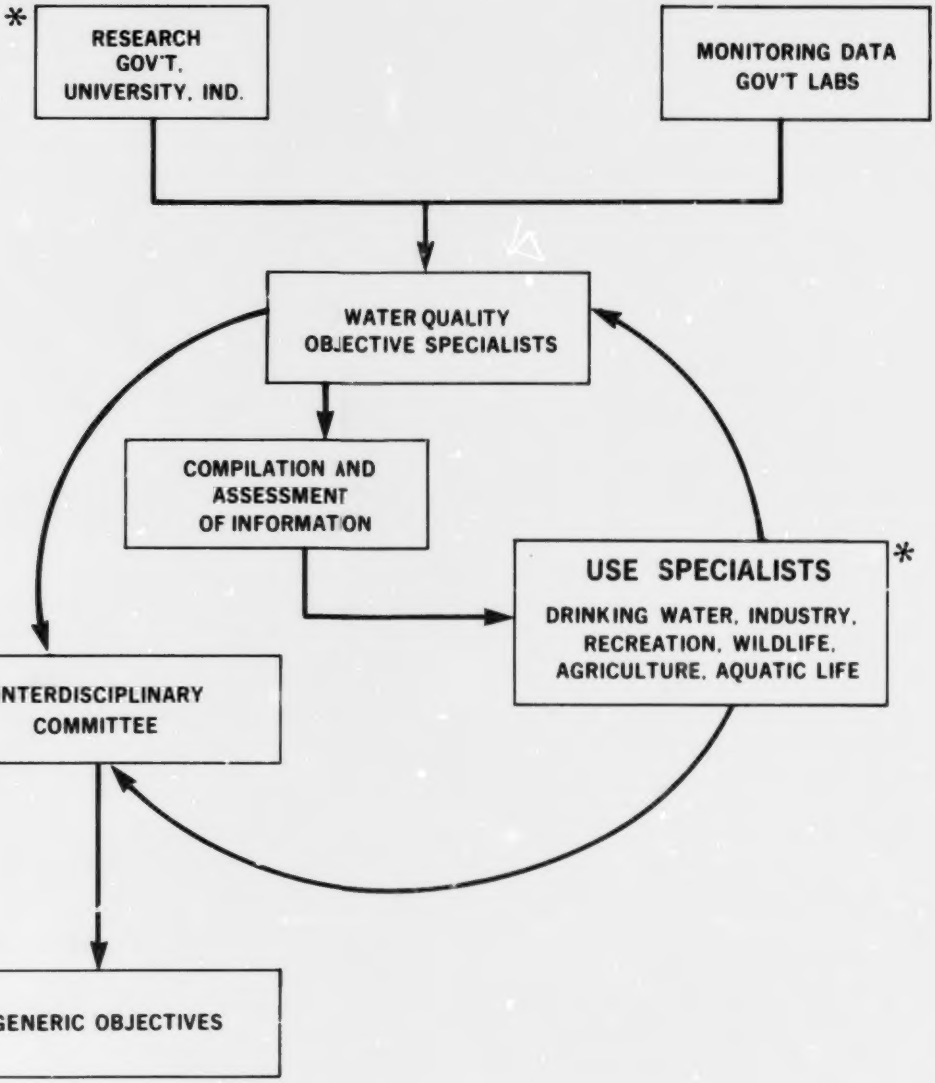
The theme of this paper is that the successful application of biological tests to industrial effluents requires coordinated effluent, receiving zone, and ambient monitoring. End-of-pipe tests are a powerful screening and regulatory tool, but they are hard to defend in the absence of in-situ biomonitoring studies. Such studies should include work on the environmental chemistry, persistence and bioaccumulation potential of the chemical constituents of effluents. Some of the case studies below illustrate these points very clearly.

2.2 Biological Testing for Water Quality Objectives

One element in the restoration and protection of water quality for specific uses is the development of water quality objectives. Each water use has a different requirement for water quality and reliable scientific facts are needed to determine what these requirements are.

The process of developing objectives in Canada is shown in Figure 1. Research information is gathered from government, university, and industrial sectors and linked with water quality monitoring data. This information is assessed and draft objectives are prepared. The objectives are reviewed critically by specialists responsible for fisheries, agriculture, drinking water etc. At this stage the objectives are generic and must be further evaluated before they can be applied to a specific water management area. Figure 2 shows that the generic objectives are evaluated in the light of present and future water uses, waste treatment technology, existing regulations, socio-economic and policy requirements, and public input. The process leads to recommendations for site specific water quality objectives for the water management area. The final step is the formal adoption of the recommended objectives by the governments and agencies concerned, and their use in choosing appropriate effluent limits.

Objectives for aquatic life are as stringent as possible. They are established from critical reviews of exposure data, data from relevant acute and sublethal aquatic toxicity tests, and if possible, knowledge of the key properties of the ecosystem being protected.


RESEARCH
GOV'T,
UNIVERSITY, IND.
MONITORING DATA
GOV'T LABS
WATER QUALITY
OBJECTIVE SPECIALISTS
COMPILATION AND
ASSESSMENT
OF INFORMATION
USE SPECIALISTS
DRINKING WATER, INDUSTRY,
RECREATION, WILDLIFE,
AGRICULTURE, AQUATIC LIFE
INTERDISCIPLINARY
COMMITTEE
GENERIC OBJECTIVES

* STAGES AT WHICH BIOLOGICAL INFORMATION IS USED

Figure 1 Process for developing water quality objectives in Canada.

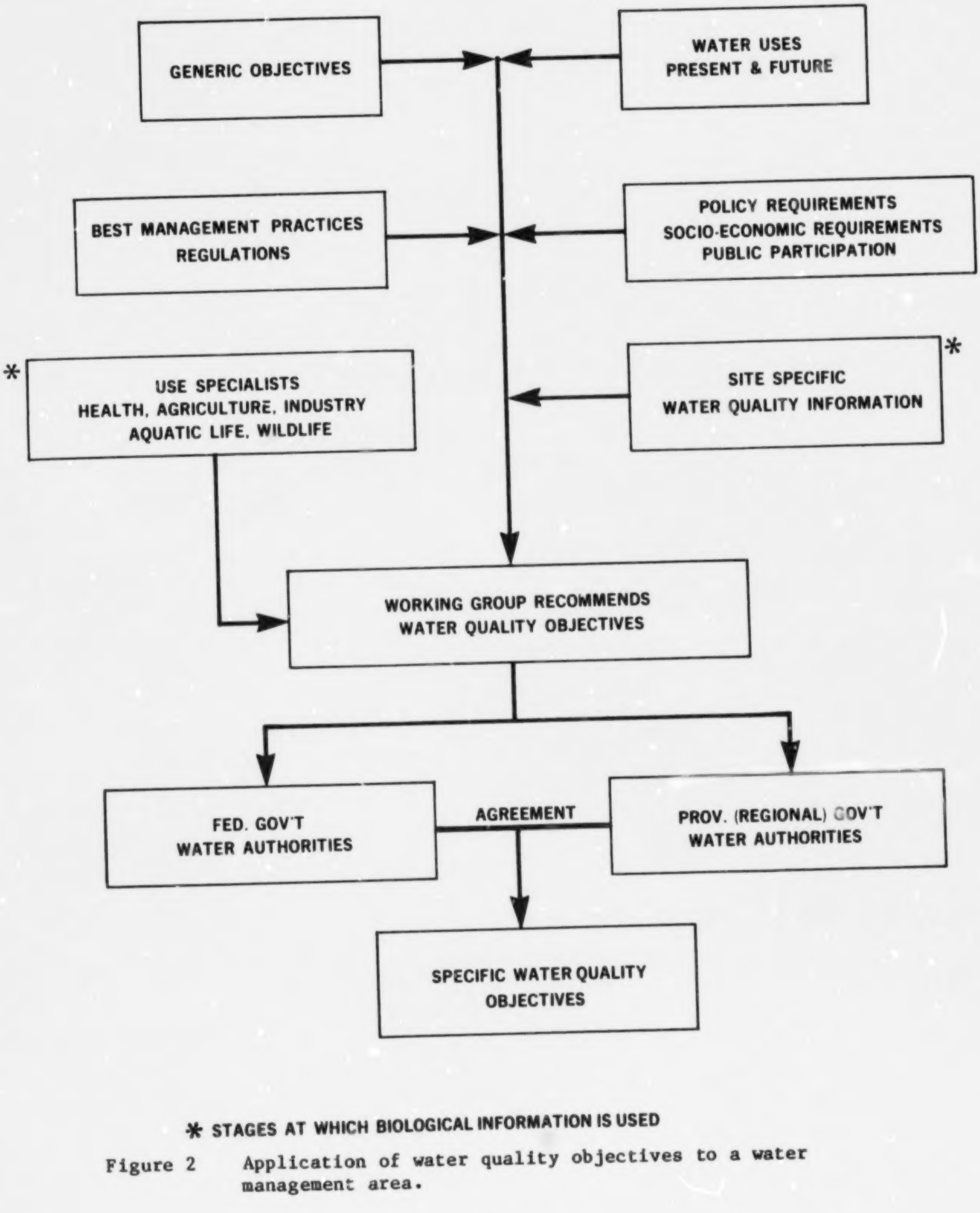

GENERIC OBJECTIVES
WATER USES PRESENT & FUTURE
BEST MANAGEMENT PRACTICES REGULATIONS
POLICY REQUIREMENTS SOCIO-ECONOMIC REQUIREMENTS PUBLIC PARTICIPATION
* USE SPECIALISTS HEALTH, AGRICULTURE, INDUSTRY AQUATIC LIFE, WILDLIFE
SITE SPECIFIC WATER QUALITY INFORMATION *
WORKING GROUP RECOMMENDS WATER QUALITY OBJECTIVES
FED. GOV'T WATER AUTHORITIES
AGREEMENT
PROV. (REGIONAL) GOV'T WATER AUTHORITIES
SPECIFIC WATER QUALITY OBJECTIVES
* STAGES AT WHICH BIOLOGICAL INFORMATION IS USED

Figure 2 Application of water quality objectives to a water management area.

In many instances data are not available for determining "safe" and "unsafe" levels of specific materials. Hence application factors are used which "provide a way of predicting a safe level which is not known from a median lethal concentration which is known" (39). In the case of pollutants that bioaccumulate, a factor of 0.01 is used. Where there is no evidence of persistence or bioaccumulation but signifiant chronic effects occur at low concentrations, 0.05 is used. When the lowest level in a chronic test is judged not to be serious, an application factor of 0.2 of the 96-hour LC50 is used. Ultimately, the application factor is chosen based on best scientific judgement. The final objective is chosen using the estimates of "safe" levels, the opinions of the water users, and the properties of the water body.

At present generic objectives are being prepared for selected priority chemicals using the process in Figure 1. Site specific objectives are in place or are proposed for several specific water management areas in Canada (the Poplar River in Saskatchewan, the Ottawa River, between Ontario and Quebec, the Saint John River Basin in New Brunswick, the Flathead River in British Columbia, and others).

2.3 Biological Tests for Routine Ambient Monitoring

The approach and practice of using biological information is more developed for setting water quality objectives than for routine monitoring. Biological monitoring can provide knowledge of adverse biological effects at pollutant concentrations below analytical capabilities, knowledge of joint effects of chemicals on biota and knowledge of various measures of the health of aquatic ecosystems.

Biological methods are being incorporated into water monitoring strategies in Canada. As techniques improve, they provide more reliable and efficient estimates of water quality problems and provide a basis for maintaining or restoring system integrity. Biomonitoring of water quality has two specific objectives: (1) to describe the presence, levels and distribution of contaminants within the aquatic system; and (2) to assess the health of aquatic systems with respect to stress.

Biomonitoring is a means of assessing the structure and function of aquatic systems. Structural biomonitoring includes population or community analyses using diversity and related indices and detection of the introduction or loss of species within the system. It can be used to assess the effects of stresses on the system. When stress is large, the structure will change. Functional biomonitoring techniques include general forms of bioassay (for acute and chronic toxicities) and other techniques based on incidence of tumors, deformities, disease and reproduction rates. This results in a static assessment i.e. how a dynamic system is functioning at one moment in time. These static assessments, together with dynamic assessments, complement the functional biomonitoring activities.

There is a need for biological indicators that are resident, easy to find, act as bioaccumulators and bioconcentrators, provide a measure of exposure to and effect of toxic substances, and provide information on causative agents. One choice of organisms for functional biomonitoring is young of the year planktiverous fish such as spottail shiners, yellow perch, and long nose dace. Two or more species may be monitored at selected sites for comparison. Specimens are obtained when fat concentrations are maximum. Data are used for determining year to year changes in water quality, comparison of levels among sites, and trends. Several functional assessments (e.g. adenylate energy charge, productivity) should be applied together.

Currently, Canada is exploring various ways of conducting such monitoring and is considering implementing a national biomonitoring strategy.

The assessment of biological communities exposed to chemical stress requires sampling spread over time. It is known that particular species or community assemblies are sensitive to environmental stresses. Structural biomonitoring is difficult to conduct. Assessments of a population or community structure, and even estimates of abundance are difficult because of the temporal and spatial variability encountered. Theoretically, structural biomonitoring is confronted with many controversies. For example, Cairns (5) concluded that a diversity index is probably the best single means of assessing biological integrity whereas Hulbert (18) and Goodman (16) concluded that such assessments had no obvious biological meaning.

Hellawell (17) considered the choice of organisms for structural biomonitoring and recommended both algae and macroinvertebrates. Both groups have been successfully used (references 34 and 41, respectively) to indicate stress on ecological systems directly related to water quality. Artificial substrates have been used, and sampled at regular intervals throughout the growth cycle of the organisms investigated. Relative estimates of abundance, composition and distribution (temporal) can be made rapidly. Descriptive analytical methods such as canonical distributions and cluster analysis may be used (15).

3. CASE STUDIES

Six studies are discussed below with the Saint John River and Canagagigue Creek presented in detail.

3.1 The International Waters of the Saint John River, New Brunswick

Results from a monitoring program, at source and in the upper Saint John River, are presented. A large sulphite pulp and paper mill located at Edmunston, N.B., is the only major source of industrial contamination in this stretch of river, which forms the boundary between Canada and the United States for about 100 km. One interesting feature of the mill is that the pulp is produced in Canada and sent in a pipeline across the river to a paper making facility in the United States. Wastewater from both operations is treated and discharged on the Canadian side into the Saint John River.

Effluents from pulp and paper industries in Canada are regulated jointly by Canada and the provinces. The federal regulations (9) specify standards based on best practicable technology for the industry as a whole. Effluent discharge permits for individual mills are issued by provincial governments taking site-specific factors into account, but must be at least as stringent as the federal requirements. The mill at Edmunston operates under a permit issued by Environment New Brunswick, which limits biochemical oxygen demand (BOD) and suspended solids.

The federal regulations present limits for BOD, suspended solids and toxicity. The toxicity requirement specifies that a mixture of 65% effluent and 35% water must be non-lethal to indigenous fish in a four-day, continuous-flow bioassay. It was Environment Canada's first aquatic toxicity requirement in a regulation, and it was used to reduce toxicity by waste treatment, not to demonstrate any direct relationship between effluent toxicity and toxic effects in the receiving waters.

The pulp mill near Edmunston has been in operation since 1918. In the mid 1970's large scale changes were implemented to control wastewater discharges. The mill's secondary treatment facility has a capacity of approximately 90,000 m^3/day; an effluent discharge higher than this results in overflow directly into the Madawaska River, a tributary of the Saint John River. The treated effluent is discharged from the secondary treatment facility into the Saint John River.

Prior to July, 1980, the volume of waste overflow was not measured but was estimated at approximately 75,000 m^3/d. Since 1 the mill operators have implemented a number of process changes to reduce the volume of effluent and since 1981 they have been successful in reducing the overflow almost completely.

The mill operators also implemented a waste liquor neutralization process in May of 1981. The waste liquor neutralization process causes volatile compounds to condense and settle out of the wastewater prior to its release. It reduced the treated effluent BOD considerably, from 17,660 kg/d in Sept. 1980 to 10,300 in Sept. 1981. By 1982 the BOD levels were meeting the summer limit of 7,260 kg/d for most of the summer months. Prior to 1981 all tested effluents had been deemed toxic according to the regulations. Effluent characteristics from 1975-1983 are shown in Table 1.

Objectives for the Saint John River have been set for protection of aquatic life and human swimmers (21). To protect aquatic organisms, long-term management objectives were developed for several parameters. These are numbers which should be met on an annual or seasonal basis but which at times may be exceeded. Results from grab samples can be compared with alert level numbers to identify immediate problems.

The long-term management objectives for many non-accumulative toxicants were set at one-tenth of the 96-hour LC50. This is a standard use of application factors developed in the early 1970's (39). Generally, it assumes that increases in concentration above the management limits will lead to slow sublethal effects, gradually reaching the lethal point as the concentration and the length of exposure increase.

The criteria by which guidelines for alert levels were developed were based on comparison and selection of LC50's and maximum acceptable toxicant concentrations provided in the literature.

The specific objectives do not take into account the effects of the interactions between polluting chemicals, physico-chemical conditions and aquatic organisms in the Saint John River. A major weakness of current water quality objectives is their inability to incorporate defensible predictions of joint toxicity for more than a few substances under ambient aquatic conditions.

A monitoring program (19) for the Saint John River was begun in 1978. The design of the program is presented in Fig.3. The most important feature of the program is the sequence of decisions based on each set of data, rather than activities of the sampling program itself.

The program relies on two types of river and effluent monitoring. The first is routine sampling of ambient water quality and effluents. The second involves intensive short term surveys of water quality and effluents, and intermittent activity directed toward answering specific questions.

Inputs to the monitoring scheme are events (eg. a review of historical data, an anticipated impact from a new development or an observed water quality problem) which would prompt an assessment of the status of the river quality with respect to the objectives. Outputs of monitoring are recommendations to management on the control of sources and the suitability of parts of the river basin for certain uses.

The major problem associated with the pulp mill effluent at Edmunston is BOD. The ambient management objective for dissolved oxygen (DO) is at least 80% saturation and the alert level is 5 mg/L. When the effluent BOD declined between 1980 and 1981, a study was conducted to determine the effect of the improvement on DO levels in the river.

Table 1 Historical characteristics of effluent data from the pulp and paper mill at Edmunston, New Brunswick.

Products: Bleached and unbleached sulphite pulp, groundwood pulp and paperboard

Allowable Limits:	BOD	Suspended Solids
Environment New Brunswick	7,260 kg/d July & Aug.	10,900 kg/d
Environment Canada	67 kg/tonne (73,700 kg/d)*	9.9 kg/tonne (10,900 kg/d)*

Year	Toxicity Test**	Avg BOD_5 kg/d	Avg BOD_5 July & Aug. kg/d	Suspended Solids kg/d	Avg Production tonnes/d	Avg Flow m^3/d
1975	May: raw LC50 range 10-18% treated LC50 range 36-42% Aug: raw LC50 18% treated LC50 29% Fail	*** Not Determined (N.D.)	*** N.D.	*** N.D.	N.D.	***
1979	Aug: LC50 29% Fail	*** N.D.	*** N.D.	*** N.D.	770	*** N.D.
1980	N.D. ****	18,000	23,000	9,500	770	112,000
1981	Aug: LC50 100% Pass	19,000	7,900	13,400	860	107,000
1982	N.D.	13,000	7,100	8,600	810	92,000
1983	July: Pass	13,000	7,700	9,400	830	97,000

* Assuming average production of 1100 tonnes/d.

** 96 hour LC50 on rainbow-trout, using 8 or 24 hr composite samples
Pass or Fail is determined using Pulp and Paper regulations

*** Before July, 1980, the volume of overflow was not measured

**** Toxicity tests were not performed when it was known that the effluent would fail

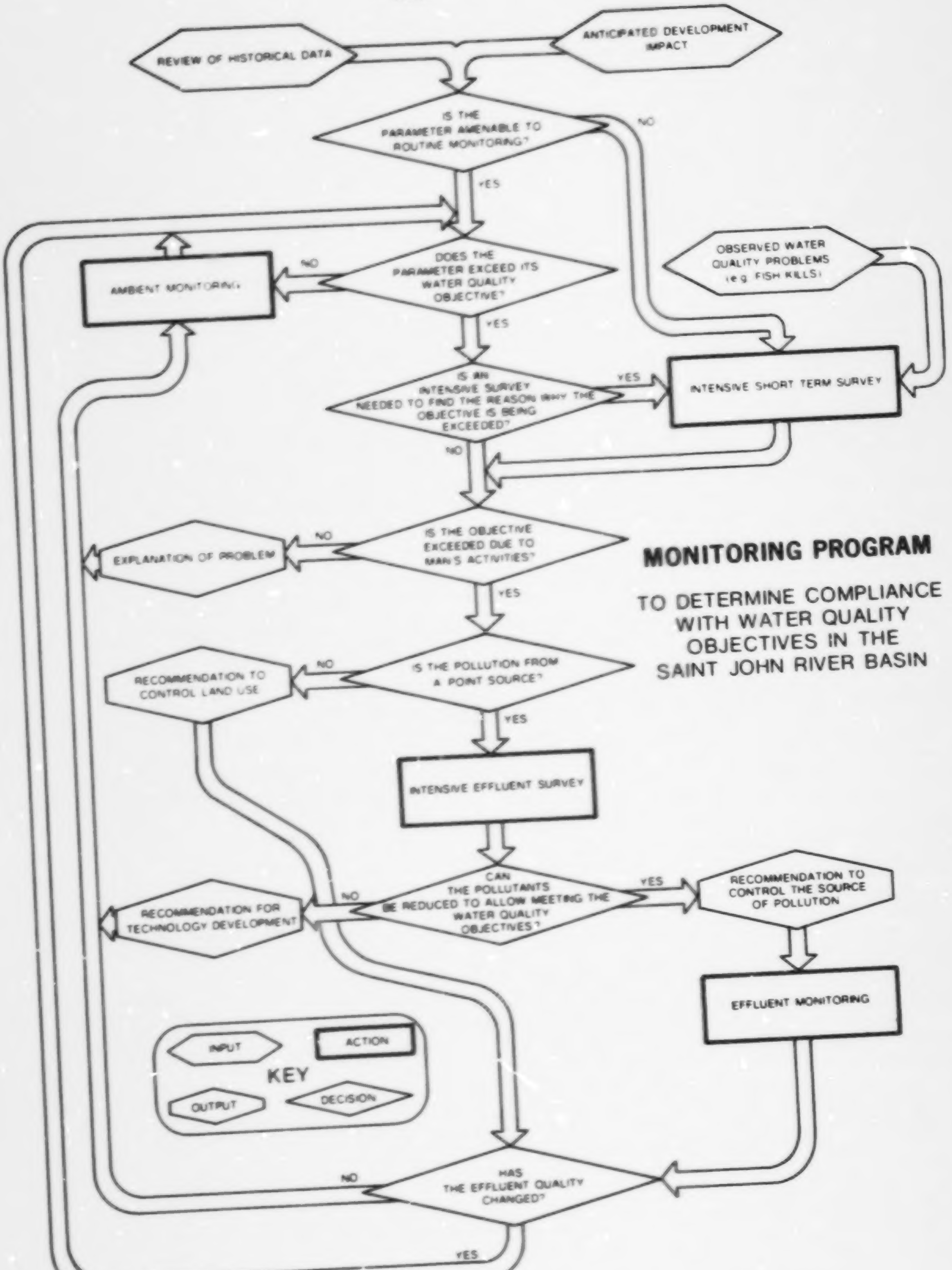

REVIEW OF HISTORICAL DATA
ANTICIPATED DEVELOPMENT IMPACT
IS THE PARAMETER AMENABLE TO ROUTINE MONITORING?
NO
YES
OBSERVED WATER QUALITY PROBLEMS (e.g. FISH KILLS)
AMBIENT MONITORING
NO
DOES THE PARAMETER EXCEED ITS WATER QUALITY OBJECTIVE?
YES
IS AN INTENSIVE SURVEY NEEDED TO FIND THE REASON WHY THE OBJECTIVE IS BEING EXCEEDED?
YES
INTENSIVE SHORT TERM SURVEY
NO
EXPLANATION OF PROBLEM
NO
IS THE OBJECTIVE EXCEEDED DUE TO MAN'S ACTIVITIES?
YES
MONITORING PROGRAM
TO DETERMINE COMPLIANCE WITH WATER QUALITY OBJECTIVES IN THE SAINT JOHN RIVER BASIN
RECOMMENDATION TO CONTROL LAND USE
NO
IS THE POLLUTION FROM A POINT SOURCE?
YES
INTENSIVE EFFLUENT SURVEY
RECOMMENDATION FOR TECHNOLOGY DEVELOPMENT
NO
CAN THE POLLUTANTS BE REDUCED TO ALLOW MEETING THE WATER QUALITY OBJECTIVES?
YES
RECOMMENDATION TO CONTROL THE SOURCE OF POLLUTION
EFFLUENT MONITORING
INPUT
ACTION
KEY
OUTPUT
DECISION
NO
HAS THE EFFLUENT QUALITY CHANGED?
YES

Figure 3 **Monitoring program to determine compliance with water quality objectives in the Saint John River Basin (19).**

A baseline study of DO was undertaken during July and August 1980 at 13 stations (Fig. 4) (20). During this time the pulp mill was discharging approximately 18,400 - 27,700 kg/d BOD. Dissolved oxygen ranged from a low of 5.7 mg/L (67% saturation) near Grand Falls to a high of 10.4 mg/L (100% saturation) above Edmunston. Between Edmunston and the St. Leonard-Van Buren area, the management objective of at least 80% saturation was generally met. Below this the geometric mean DO was considerably less than 80% saturation.

Another survey was carried out in the summer of 1981 (22), at a time when the mill discharged approximately 7,000-8,000 kg/d BOD. Again, no sample from the river was less than 5 mg/L. In addition, the average DO levels (and most individual samples) met the management objective of at least 80% saturation. The lowest level, 5.8 mg/L, was similar to the 1980 low and occurred at the same station at Grand Falls. Saturation values at this point were generally 68-75%, an improvement over the previous year when saturation values were 57-74%.

Over a one-year period, the mill was able to change its process and treatment practices to reduce BOD and to produce a non-lethal effluent. These changes were reflected in improved conditions in the river. The trout lethal toxicity test was useful in identifying the problem. However, the trout test itself did not identify BOD as the property of concern. In their efforts to reduce effluent volume the mill operators reduced BOD levels. This also reduced the lethality of the effluent.

The trout lethality test is not useful for predicting problems with persistent pollutants which do not exert acute lethal effects. The pulp mill at Edmunston may indeed be discharging low levels of persistent substances which will only be detected by sensitive chemical and biological testing at source and in the river. If routine ambient monitoring is restricted to chemical analysis of the water without involving analysis for chemical residues in biota and sediment, and for subtle biological changes, persistent lipophilic toxicants may go undetected until obvious environmental changes occur.

3.2 Canagagigue Creek

The Canagagigue Creek Field Study examined the fate and impact of synthetic organic contaminants in a small stream (Fig.5). The section of creek under study extends about 13 km from the Woolwich Reservoir, through the town of Elmira, Ontario, to the confluence with the Grand River.

The Elmira Water Pollution Control Plant (WPCP) produces the only direct discharge to the creek. However, it receives about 50% of its BOD from a specialty chemicals plant whose products include rubber additives, fungicides and poly-alpha olefins. Effluent from

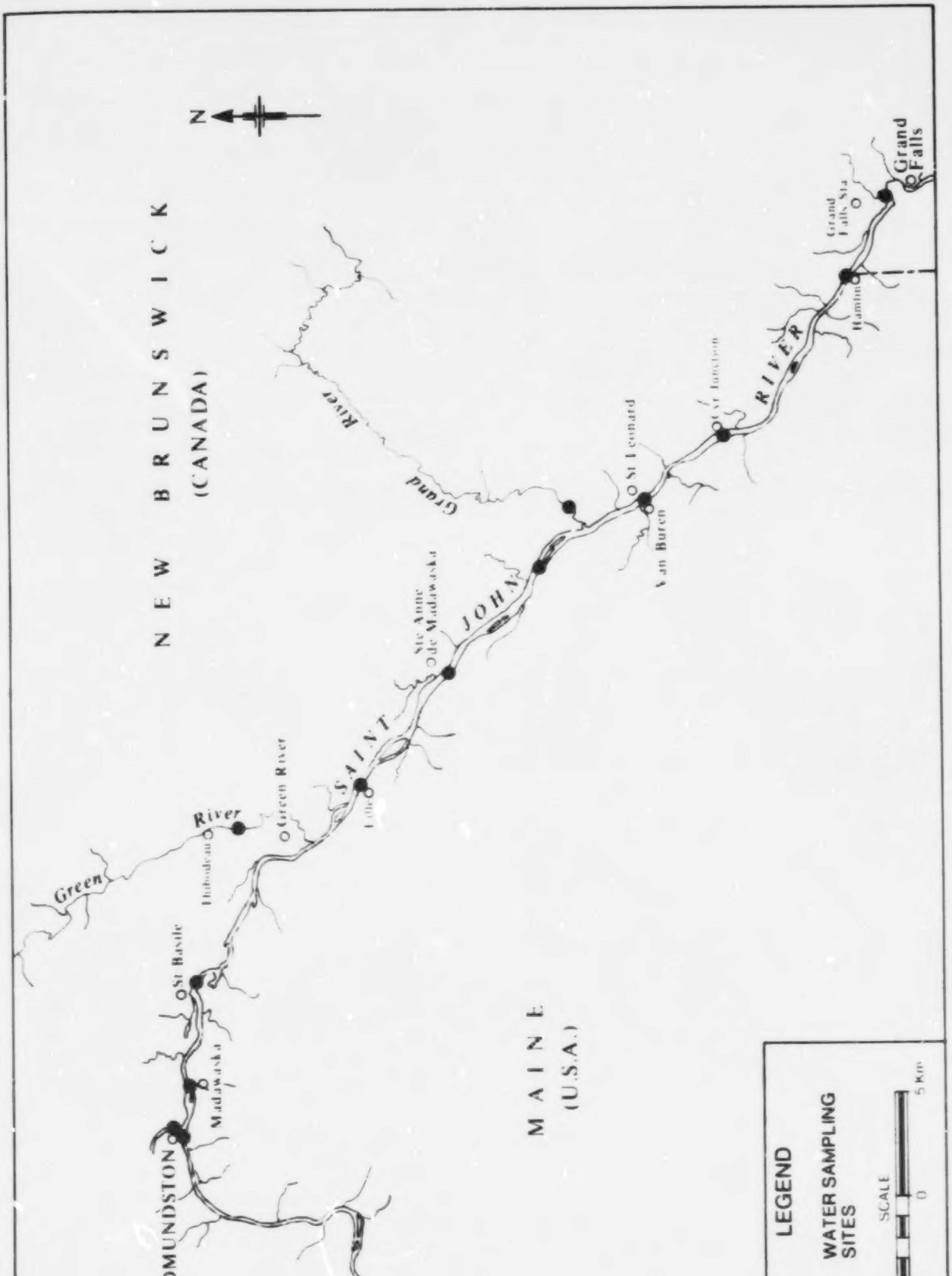

NEW BRUNSWICK
(CANADA)
MAINE
(U.S.A.)
N
EDMUNDSTON
Madawaska
St Basile
Green River
Thibodeau
Green River
Lille
SAINT JOHN RIVER
Ste Anne de Madawaska
Grand River
St Leonard
Van Buren
Hamlin
Grand Falls Sta
Grand Falls
LEGEND
● WATER SAMPLING SITES
SCALE
5
0
5 Km

Figure 4 Water sampling stations on the international waters of the Saint John River Basin.

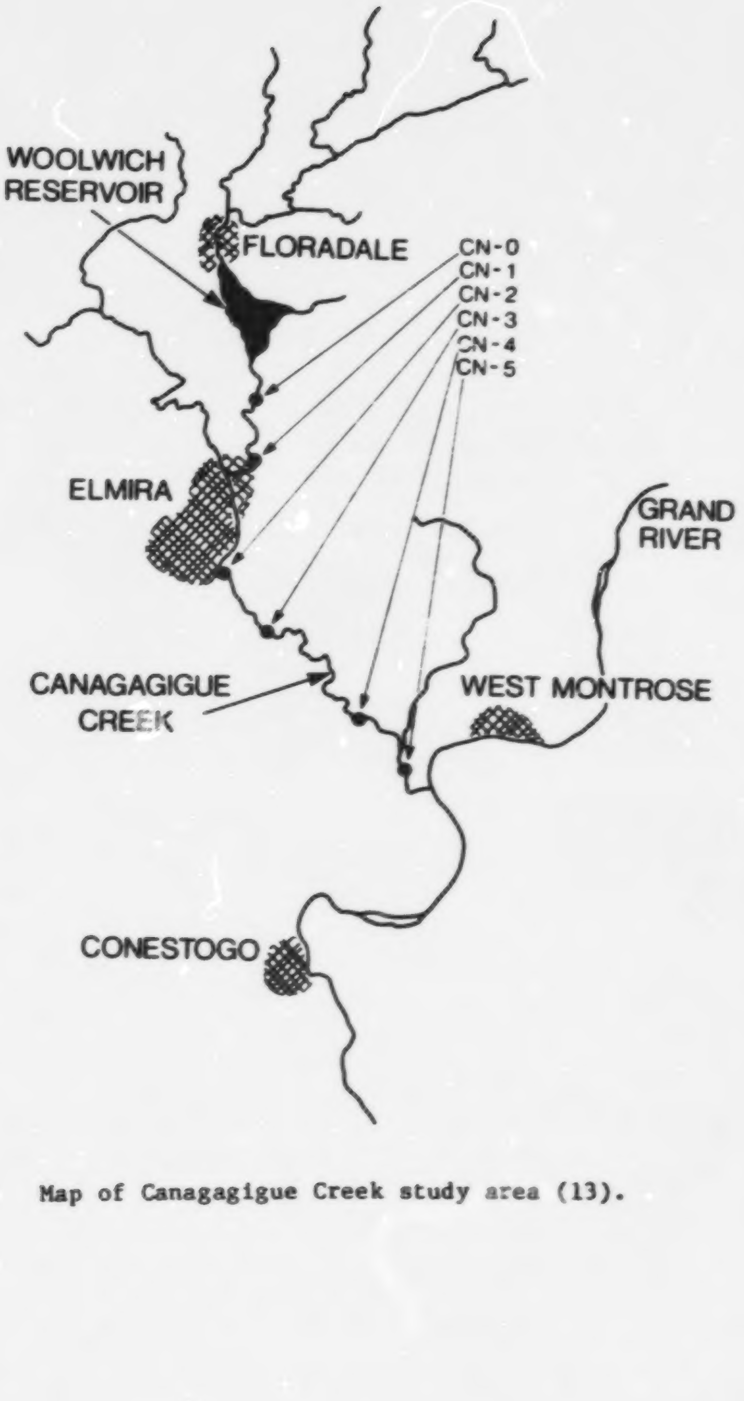

WOOLWICH RESERVOIR
FLORADALE
CN-0
CN-1
CN-2
CN-3
CN-4
CN-5
ELMIRA
GRAND RIVER
CANAGAGIGUE CREEK
WEST MONTROSE
CONESTOGO

Figure 5 Map of Canagagigue Creek study area (13).

Table 2 Acute toxicity of effluents entering Canagagigue Creek, Ontario (32).

	Sept.76	April 77	July 81	July 82
Town water	---	---	non-lethal (7.8)[2]	---
Chem. Co.				
- cooling water	---	---	non-lethal	non-lethal
- south lagoon	---	---	82%	---
- influent (carbon filter)	6.0%	3.9%	---	---
- effluent to WPCP	45%	22%	---	100%
Elmira WPCP				
- sewage alone	---	---	40%	---
- influent	---	58%	---	---
- effluent before Clorination	non-lethal	---	54%	52%
- final effluent, chlorinated	38%	59%	---	non-lethal

1 96-h LC50 for fingerling rainbow trout
2 grab samples

the chemical plant receives preliminary treatment in a series of lagoons followed by activated carbon filtration and pH adjustment before discharge to the WPCP.

The Elmira WPCP final effluent, which is chlorinated, is a major source of nutrients (ammonium and phosphate), conservative ions (sulphate and chloride) and organic contaminants (primarily phenols). A summary of 96 hour static toxicity tests exposing rainbow trout to grab samples of various WPCB effluents is presented in Table 2. Toxicity data are sparse, but it appears that the final effluent had improved in 1982. This may have been due to the initiation of a wetlox system in the chemical plant, which destroys organic chemicals at high temperatures and pressures (Tom Singh, pers. comm.). The field study consisted of three phases:

(a) Identification of the major synthetic chemicals in the stream that were appropriate for further study and a qualitative examination of stream biology to determine ecologically significant zones and select appropriate sampling sites.

(b) Examination of environmental components in the stream, to determine the factors controlling bioaccumulation and degradation of the identified contaminants, and to search for effects of these contaminants in the stream.

(c) Laboratory verification of the controlling factors and effects identified in (b).

The ecology of Canagagigue Creek (12) was assessed by collecting fish and benthos seasonally from six sites (CN-0 to CN-5 in Fig. 5) and determining species abundance and diversity. In general, fish and benthos demonstrated a trend of markedly decreased abundance and species diversity immediately downstream from Elmira relative to the upstream sites. Recovery from the environmental stress imposed by discharges at Elmira was demonstrated by high species abundance and diversity at the farthest downstream site.

The effect of the WPCP is clearer if individual species are considered. Figure 6 illustrates the population distributions, on a percentage basis, of several organisms in the creek. The disappearance of elmid beetles, hydropsychid caddis larvae and sphaerid clams from sites immediately downstream from Elmira indicate that they are unable to tolerate conditions in this portion of the creek. On the other hand, blackfly, oligochaete and dancefly (Hemerodromia) populations responded favourably downstream, indicating that they are tolerant of organic pollution. Although the same patterns were observed in the fish census, the fish distributions must be interpreted with caution because physical barriers and migration from the Grand River may have affected the results. The benthic community structure is probably a better indicator of water quality in the stream.

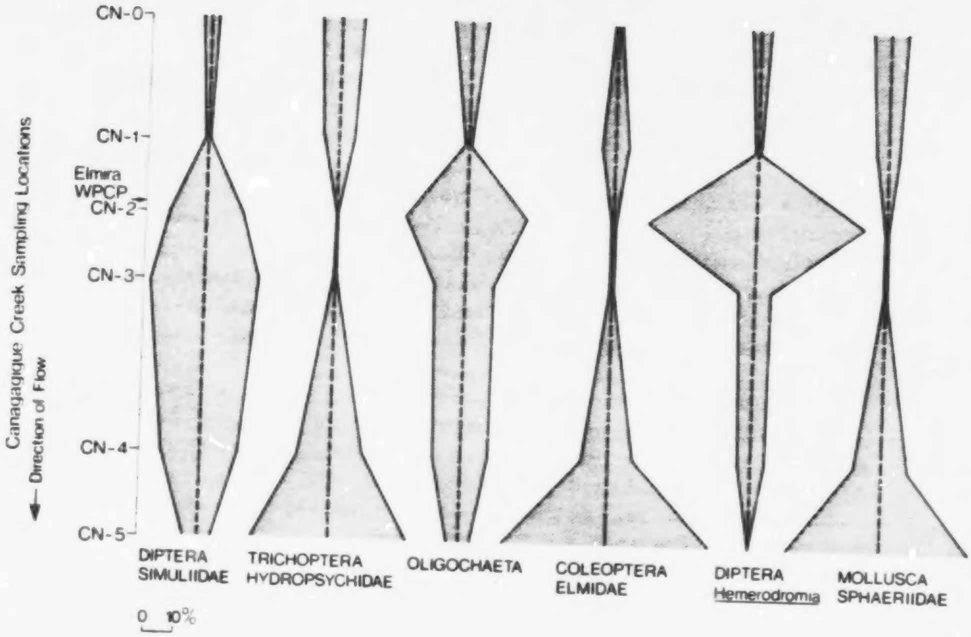


Figure 6 Population distributions of some of the dominant benthic organisms from Canagagigue Creek in 1980.

The chemical survey revealed that Elmira was a source of chlorophenols (CPs), benzothiazoles and lindane to the stream. The predominant chlorophenols were 2,4-dichlorophenol (2,4-DCP), 3,4-dichlorophenol (3,4-DCP), and 2,4,5-trichlorophenol (2,4,5-TCP). The source of these CPs is a disused chemical waste disposal site on the grounds of the chemical plant. This plant had manufactured CPs and chlorophenoxyacetic acid herbicides up to 1969. The predominant benzothiazoles were benzothiazole (BT), 2-(methylthio)benzothiazole (MMBT), 2-(methylsulfonyl)benzothiazole and 2-(methylsulfinyl)-benzothiazole. None of these compounds are commercial products of the chemical plant although benzothiazoles, including 2-mercaptobenzothiazole (MBT), its disulfide DBBT and several sulfenamines, are current products. Both BT and MMBT have previously been observed in environmental samples but their source has not been identified.(4)

With the co-operation of the chemical company, water samples from their wastewater treatment lagoons, before and after activated carbon treatment, and wastewater from the thiazole synthesis were obtained and examined for MMBT and its oxidation products. These compounds were not detected in the samples, indicating that they are not likely formed as by-products in the thiazole synthesis. Currently, it is felt that MBT is methylated in the WPCP by bacterial action and the resulting MMBT is oxidized by the chlorine used in the disinfection of the WPCP effluent.

In addition, acidic compounds were identified which were products of the degradation of alkylphenol non-ionic surfactants occurring in the sewage plant,(10). These surfactants are used as dispersants, dye levelers and detergents in a variety of industrial applications. The source of these compounds to the sewage plant was not investigated.

The second phase of the project has concentrated mostly on CPs because they are the most prominent biologically active compounds observed in Canagagigue Creek and because sensitive methods are available for their analysis. Intensive sampling of the creek was performed to examine the environmental distribution and disappearance of these compounds. The disappearance kinetics of 2,4- and 3,4-DCP were first order with respect to either distance or time of flow.(11) As shown in Table 3, both CPs disappeared at approximately the same rate with little seasonal variability in the rate constants. The lack of seasonal trends along with the observance of first order kinetics led to the conclusion that the rate controlling factor was not biological. The disappearance may be due to degradation within the biofilm covering the bottom substrate, at a rate controlled by diffusion to the biofilm surface.

The distribution of CPs is of considerable interest. Sediment traps were placed in the stream and sampled monthly to collect

Table 3 Disappearance rate constants (K_D, hr^{-1}) and halftimes ($T_{\frac{1}{2}}$, hr) for 2,4-DCP and 3,4-DCP, 1981.

	2,4-DCP		3,4-DCP	
Date	K_D	$T_{\frac{1}{2}}$	K_D	$T_{\frac{1}{2}}$
April 7	.186	3.7	.105	6.6
May 5	.172	4.0	.166	4.1
June 2	.164	4.2	.151	4.6
July 7	.206	3.3	.175	4.0
August 4	.230	3.0	.253	2.7
Sept 1	.347	2.0	.294	2.4
Sept 29	.072	9.6	.078	8.9
Dec 8	.145	4.8	.164	4.2
Average K_D	$.190 \pm .079\ hr^{-1}$		$.173 \pm .064\ hr^{-1}$	

suspended solids for CP analysis. These results, along with a comparison of filtered and unfiltered water, revealed that the CPs were transported almost exclusively in the water phase and not on solids. The pKa's of these compounds were about 7 and the stream pH averaged about 8. Most CPs were in the ionized form in the stream and therefore less likely to partition into the non-aqueous phases.

The levels of CPs in biota were analysed. Among the fish, rock bass, which is the chief predator at most of the sites, contained the highest levels.(12) A relationship between the CP levels in fish and water was apparent with both compartments exhibiting a mid-summer minimum. With the exception of Hirudinea (leeches), a similar trend was noted in the benthos. The CP concentrations in most organisms appeared to exist in a dynamic equilibrium with the water concentrations. However, leeches accumulated very high levels of CPs, usually orders of magnitude above those found in other benthos. Table 4 gives a comparison of typical levels of CPs in several organisms. There are four abundant species of leech in Canagagigue Creek: Dina dubia, Erpobdella punctata, Glossiphonia complanata and Helobdella stagnalis. Dina dubia had the highest levels of CPs but it is not a common species at other locations in Canada. Both Erpobdella punctata and Helobdella stagnalis are more widespread and accumulate significantly high levels. This suggests that leeches be used as bioindicators of contamination of surface waters by phenols(26).

The benthos was significantly affected by several spills of nonylphenol occurring in the stream. During 1981, the benthic community was virtually eliminated in the reach downstream from Elmira and took some months to recover.(13) This occurrence points out the sensitivity of field collections as a survey or monitoring tool; they are restricted to those organisms that are present at the time of sampling and there is no guarantee that a suitable species will be collected or will remain unaffected by unpredicted events. This is especially true for univoltine organisms in areas receiving sporadic toxic discharges where organisms may be absent due to insufficient time to recover from a toxic incident but all traces of the responsible chemical have disappeared from the system.

Two studies were initiated in 1982 to look for effects of Canagagigue contaminants on fish. Survival of the early life stages of the common shiner (Notropis cornutus) at CN-3 was compared with survival at a site upstream between CN-0 and CN-1. Studies were carried out in hatchery troughs located in trailers on the stream bank. Feed water for the troughs was continuously pumped from the stream, filtered with a sand filter and oxygenated. Survival to hatch was 80% at both sites but survival to the critical swim-up stage was reduced from 69% upstream to 49% downstream. These results showed that conditions downstream from Elmira may reduce reproductive success of indigenous fish. In another experiment, rainbow trout fingerlings were reared for six weeks at the two sites. No differences in mortality or growth rates between sites were observed, in

Table 4 2,4,5-Trichlorophenol levels in Canagagigue Creek biota and water at site CN-5 on four occasions in 1981.

	2,4,5-TCP concentration (ppb)			
	June 2	July 9	Sept.30	Nov. 4
Leeches (*Erpobdella punctata*)	4524	1046	-	-
Leeches (*Dina dubia*)	-	1356	-	4917
Leeches (*Glossiphonia complanata*)	-	-	710	640
Leeches (*Haemopsis gradis*)	-	-	1110	-
Aquatic worms (*Oligochaeta*)	561	-	-	894
Caddisfly larvae (*Hydropsychidae*)	19	-	7	26
Caddisfly larvae (*Pycnopysche sp.*)	185	34	-	-
Cranefly larvae (*Tipulidae*)	90	-	ND	1
Fishfly larvae (*Nigronia sp.*)	18	-	ND	-
Diving beetles (*Agabus sp.*)	1	-	-	-
Limpets (*Ferrissia sp.*)	5	-	-	-
Fingernail clams (*Sphaeriidae*)	ND	-	-	-
Dragonfly larvae (*Anisoptera*)	8	-	8	2
Damselfly larvae (*Zygoptera*)	ND	-	9	46
Snails (*Physa sp.*)	-	-	14	36
Water pennies (Psephenidae)	-	-	2	1
Water	.067	.036	.041	.015

contrast to results of toxicity tests (96 hour LC50s) which showed that the final effluent from the Elmira WPCP was often quite toxic (Table 2).

As shown in the figure, the ecological effect of the WPCP effluent was most readily seen in the population distributions of individual benthic species. The diversity index was less useful as an indicator of ecological conditions. For example, although more taxa were found at CN-1 than CN-0, the diversity index value was lower. The infrequent appearance of new taxa was apparently masked by the index. There was also some difficulty in using the index with information from several sources. Unless all organisms are consistently identified to the species level, the value calculated for the diversity index will be understimated, making site to site comparisons difficult.

The difficulty in using biomonitor species to identify problems was also apparent. If a system is grossly polluted or is subject to toxic incidents, the choice of available organims as bioindicators is limited. Leeches appear especially promising since they responded favourably to gross organic pollution and were most numerous in the most polluted locations. However, their ability to concentrate neutral organic contaminants in unknown.

The search for effects of Canagagigue contaminants on fish was also of interest. No effects on mortality or growth rates of rainbow trout fingerlings were noted for the water at CN-3. However, survival to hatch of common shiners was reduced at this site relative to the clean site. In addition, many benthic species were absent in this region of the creek. Thus the trout bioassay did not appear to be a good indicator of toxic effects at this site.

3.3 Saint John River - Chlorophenol Sampling in the Lower Reaches

When it was shown that leeches in Canagagigue Creek, Ontario, were accumulating chlorophenols to concentrations orders of magnitude higher than other aquatic organisms, leeches were proposed as bioindicators of aquatic contaminants(26). In order to determine whether such bioaccumulation in leeches occurred elsewhere they were collected in other river systems and analysed for chlorophenols and other organic contaminants. One of these systems was the Saint John River below Woodstock, New Brunswick, about 250 km below Grand Falls. Three sites were selected close to a known source of organic pollution or to a National Aquatic Database (NAQUADAT) station.

Seven species of leech were found with the dominant species being Erpobdella punctata, Helobdella stagnalis and Glossiphonia complanata. Leeches proved to be good indicators of chlorophenol contamination. Only three chlorophenols were detected in the water but twelve were detected in leeches from the various sites. Concentrations in the leeches were three to four orders of magnitude higher than in the water.(26) The possible sources of CPs are pulp and paper mills upstream (23).

Leeches appear to be a good indicator of chlorophenol contamination which would not be detected by measuring wastewater discharges alone or by routine water monitoring.

3.4 Niagara River, Ontario - New York

The Niagara River connects Lake Erie to Lake Ontario and receives discharges from steel, petrochemical and chemical manufacturing industries as well as from municipalities. Chemicals are also leaching from nearby landfill sites into the river.

The Niagara River Toxics Committee (NRTC) was formed in 1981 to cooperate in a joint investigation of toxic chemicals entering the river. The task before the NRTC was to assess the pollutants in the river, identify the sources, implement activities to reduce the pollution and establish procedures to monitor the effectiveness of these activities.

The following is a summary of the process used to choose the chemicals of most concern, and the effluent monitoring and ambient biological monitoring activities (31).

Using monitoring reports on the Niagara River and its tributaries, the western end of Lake Ontario and the eastern end of Lake Erie, the NRTC established a list of 261 man-made chemicals which are present in the system. The chemicals were screened into 3 groups: I) chemicals requiring immediate attention; II) chemicals requiring continuing monitoring or further investigation, and III) chemicals of minimal concern.

The screening process dictates priorities for research and indicates which chemicals (Group I) should definitely be included in any monitoring programs.

The screening mechanism had two distinct phases. The first involved separating the chemicals into two groups, those for which quantitative data existed and those for which it did not. Environmental levels that were found were compared to the objectives of participating agencies. When these were found to be greater than or equal to the most stringent objectives, the chemicals were assigned to Group I (chemicals requiring immediate attention). Those found at less than the objectives were placed into Group II.

The second phase was less straight-forward and involved the use of the 1982 Annual Report of the Committee on the Assessment of Human Health Effects (HEC) of Great Lakes Water Quality (14). Each chemical was categorized for toxicity and exposure. Also involved was a modified version of the Michigan Critical Materials Register (29). It utilizes a hazard assessment methodology which considers acute toxicity, carcinogenicity, teratogenicity, mutagenicity, persistence, accumulation and other adverse effects. Factors assessed were limited to bioaccumulation and acute aquatic and terrestrial toxicity. A numerical score referred to as an "acute effects ranking" (AER) was derived from bioaccumulation and acute toxicity data collected from a variety of sources. The score ranged from 0 to 14 (the higher numbers reflecting higher environmental significance). Where only one data element (bioaccumulation or acute toxicity) was available this was noted.

Many chemicals fell into Group II (chemicals requiring continued monitoring or further investigation) due to the paucity of data. As a result Group II was divided into 7 sub-groups.

Chemical analysis for EPA priority pollutants in grab samples from significant dischargers was carried out. Landfills in the area were also investigated to determine which ones had the potential to contribute contaminants to the Niagara River.

Ambient biological monitoring along with sediment and water monitoring was carried out in the river to assess the extent of contamination and to locate sources of contamination. The work is summarized below:

i) Spottail shiners (Notropis hudsonius) have a restricted range and were used to identify temporal and spatial trends in the river.

ii) Macroinvertebrate studies (collecting, identifying and enumerating organisms) compared the community structures in a number of locations. Comparison with results from previous years indicated an overall improvement in water quality (37). These studies did not involve tissue analysis.

iii) Algae (Cladophora) were collected from a number of sites and analysed for PCBs and metals. This has helped to identify sources of metal discharges.

iv) Clams (Elliptio comptanatus) were exposed to river water at 14 sites for three weeks and analysed for a number of contaminants. This technique has been useful in identifying sources of contamination and confirming the results of other biological monitoring.

v) Data on contamination in sport fish are extensive and showed increased levels of contamination in the lower reaches of the river (32).

The monitoring results indicate that all parts of the Niagara River ecosystem are contaminated and give approximate locations of major sources of certain chemicals. Monitoring to evaluate the effect of improved discharge control on the river should continue. Monitoring is also needed to identify further controls, and to ensure compliance with current requirements.

The NRTC will probably recommend that biomonitoring of point source discharges take place on a routine basis. Continuous flow long-term bioassays using fish or crustaceans on effluents will be carried out periodically to monitor acute and chronic toxicity. Some of the exposed individuals will be analyzed to determine which contaminants are bioaccumulated.

3.5 Tobin Lake, Saskatchewan

Tobin Lake is a man-made reservoir on the Saskatchewan River. The river above Tobin Lake receives contaminants from five major sources: agricultural runoff, petrochemical industries, pulp and paper mills, mining and municipalities. The sediments in Tobin Lake are a sink for these contaminants (3). The effluents from all industries discharging to the river system are operating under provincial licence.

Interest in Tobin Lake was prompted by the discovery of a relatively high incidence of morphological deformities in resident chironomid larvae. It was suggested that chemicals in the sediment may be responsible (3).

Samoiloff and others (36) developed methods coupling biological and chemical testing to establish and rank the contaminants in the sediments producing the greatest long-term risk. The method involved extracting contaminants from samples, fractionating the extracts into eight separate fractions on the basis of differences in solubility, using bioassay techniques to determine the relative toxicity of each fraction, and identifying the major components of the most toxic fraction.

Two bioassay methods were used in the study. The Salmonella typhimurium test was used as a standard indicator of mutagenesis while an assay using the nematode Panagrellus redivivus was used to detect lethal and sublethal (developmental, mutagenic) effects.

The P. redivivus assay indicated that the most toxic components in Tobin Lake are in fraction 2 (neutral compounds soluble in 1:1 hexane-dichloronethane) and this was confirmed by the S. typhimurium assay. The results of the assays are compared and ranked in Table 5. On the basis of the above, samples of fraction 2 were analysed chemically by gas chromatography-mass spectroscopy. Fifty-two potentially toxic compounds were found at two locations.

Although this approach does not identify the most toxic compounds present, it does identify the classes of compounds producing the greatest hazard and reduces considerably the number of compounds considered as high priority contaminants at the two sites examined in Tobin Lake. The lack of detectable toxicity in fraction 1 (neutral compounds soluble in hexane) indicates that, based on these methods, pesticides are not contaminants at these sites.

Table 5 Comparison of bacterial and nematode bioassays on fractions of Tobin Lake sediments (36).

		bacterial test		nematode test	
Fraction	Description	effect	rank	effect	rank
1	neutral hexane extract				
2	neutral hexane-dichloromethane extract	I,M	1	I,S,L	1
3	neutral dichloromethane extract	M	2	I	4
4	neutral methanol extract			M	5
5	containing strong bases			M	6
6	containing strong acids			M	7
7	hydrophilic	M	2	I,M	2
8	most hydrophilic	I,M	1	I	3

M = mutagenic
I = inhibitory
SL= semilethal

Ranking is done by assigning the most toxic fraction the lowest number

This work demonstrates that a protocol combining chemical fractionation and biological testing of environmental samples can be used to determine and rank the toxic components in a system and to localize major contributors to hazard in a real aquatic ecosystem. The nematode assay is now being used to test sediment in the North Saskatchewan River between point sources.

3.6 The Columbia River, British Columbia

The town of Trail, British Columbia, is the site of a lead-zinc smelter and refinery complex. The facility is located approximately 18 km upstream of the Canada-U.S.A. border on the Columbia River. No international water quality objectives have yet been developed for the river. However, the smelter-refinery complex discharges are subject to permit restrictions, limiting pH and concentrations of metals in effluents.

Current work on the effects of discharges on river biota include algal bioassays, measures of metals in fish tissue, and analyses of metallothionein in fish livers. This information is being gathered concurrently with effluent information in an attempt to relate conditions in the river to the effluent concentrations of contaminants. The aim is to provide information on receiving water quality so that the appropriate remedial actions may be taken.

Environment Canada, the provincial authorities and industry cooperate in the collection of effluent data; and chemical and biological in-stream information. Where possible this is being done concurrently.

A range of in-stream effects have been noted which relate to operations in the refinery complex. Periodic discharges in excess of the prescribed limits and accidental spills from time to time have resulted in lowered pH levels in the river. The pH level has not been observed to fall more than 0.4 units in the Columbia River above the confluence with the Pend d'Oreille River, which is just upstream of the international boundary. However, the highly acidic discharges may dissolve metal contaminants from sediments in the immediate vicinity of the effluent pipes.

At this time the data are being interpreted so as to relate the chemical quality of the river and effluents to the biology of the river. This information is currently being used to take remedial action and may ultimately be used to set objectives for water quality at the international boundary.

4. DISCUSSION

Both effluent and ambient biological monitoring have been conducted in Canada to determine the effluent's toxicity and to predict the impact of toxic substances in specific effluents on the aquatic environment. Effluent testing has been particularly extensive in the past 10 years. However, as seen in the selected case studies, effluent testing and ambient monitoring often have been used with varying degrees of interdependence and success. The contributions of both approaches to pollution control and their limitations are discussed.

End-of-pipe testing often makes a valuable contribution to problem identification and description, to administrative action on the problem, and to determining the effectiveness of controls. Even relatively simple bioassays (e.g. measuring fish lethality and swimming impairment) have been effectively used many times to identify serious problems and to contribute to water quality criteria. For example, in the Upper Saint John, effluent tests confirmed the source of the dissolved oxygen problem in the river and led to decisions concerning treatment. Toxicity data were used to identify the optimal oxygen levels for both the river and the effluent. A similar application has been made for many specific effluents (Table 6) and is in progress for the smelter study on the Columbia River. In contrast, the problem with chlorophenols at Canagagigue Creek was identified in the field using primarily chemical analyses, and in the lower Saint John, the chlorophenol contamination was described through the aid of a leech biomonitoring technique.

Mount (30) stated that "it is extremely important to be familiar with and clear about the specific purposes and limitations of fish toxicity tests intended for use in regulatory biomonitoring programs". Sprague (38) and others have made similar statements about toxicity tests and their applications in general. This emphasizes the importance of designing both general and specific toxicity protocols for meeting regulatory and guideline requirements for end-of-pipe and receiving water objectives. Different objectives also require different degrees of sophistication of the tests employed. For example, acute lethal tests with fish and effluents may be suitable for initial control of toxic effluents, while sensitive sublethal tests (single-species and multi-species, ranging from laboratory to field) are required for the second level of testing with effluents, ambient waters, and specific priority chemicals.

Review of the selected case studies (Table 7) shows that the various applications of biological testing may not be followed in practical situations. There are a number of reasons for this. In Canada, as elsewhere, the water sector is administered by a number of agencies representing different levels of government. This leads to both interjurisdictional and interagency considerations which may limit the scope of individual studies. Resource limitations also influence the intensity and scope of each study. Different agencies may apply their unique skills to a particular study at different times, complicating the goal of coordinated sampling and analysis.

Table 6 Some applications of end-of-pipe toxicity testing (W.R. Parker, personal communication).

Project Description	Test Type	Outputs/Achievements
Pulp & Paper: Georgia Pacific Mill in Maine.	Semi static and flow through (FT) acute lethal toxicity tests (ALTT) with Atlantic Salmon.	Data used to support cleanup of St. Croix River by US EPA and the International Joint Commission.
Metal Mining: Heath Steel Mines, N.B.	Static, FT, ALTT with rainbow trout.	Investigate the treatability (toxicity reduction) of metal mine liquid effluents.
Metal Mining: Northeastern N.B.	Lethal and sub-lethal toxicity tests with Atlantic Salmon.	To establish meaningful water quality criteria to protect the Atlantic Salmon fishery.
Pulp & Paper: AnilCanada Ltd., East River, N.S.	Static, FT, ALTT with rainbow trout and Atlantic salmon.	Investigate the effects of the final effluent on the aquatic environment.
Food processing: Fish Plants in Nova Scotia.	Static ALTT with rainbow trout and mummichog (F. heteroclitus)	Characteristics and environmental effects of fish plant wastes.
Gold Mining.	Static ALTT using rainbow trout.	Develop data on the effect of pH and hardness on the toxicity of cyanate and thiocyanate in support of regulations and guidelines development for gold mines.

Table 7 Evaluation of selected case studies.

	Upper Saint John River, N.B.	Canagagigue Creek, Ontario	Lower Saint John River, N.B.	Niagara River, Ontario	Tobin Lake Saskatchewan	Columbia River B.C.
Problem	- High BOD of pulp mill effluent. - Low dissolved oxygen in river.	- Chemicals Co. and municipal discharges.	- Chlorophenols (CPS) in the river.	- Chemical contaminants coming from many sources, mostly point-sources.	- A variety of point and diffuse discharges. - Reservoir identified as sink for contaminated sediments.	- Pb, Zn refinery discharge. - Acid and Zn spills - Maintain H_2O quality at Int'l border.
Approach - Agency Objectives, Standards	- Effluent standard. - Ambient objective.	- No objectives or standards.	- No objectives or standards.	- GLWAQ (1978). - No water qual. objective. - Effluent standards on some indus.- N.Y. & Ont.	- No objectives or standards.	- Effluent standards - pH limit for the river
Ambient Waters	- Chemical survey of DO to monitor effectiveness of controls.	- Effects on populations in the locality of the out flow. - Leeches show high levels of CP's.	- Leeches concentrate CPS above levels found in water.	- Residue analysis in tissue used to identify possible sources.	- Bacteria and nematode testing on sediment extracts.	- Algal. bioassay-fish tissue analysis, and community structure related to various levels of chemical discharges.
Source Effluents	- Trout toxicity test used to identify problem and ensure continued control.	- Trout toxicity test indicated toxic effluents from chemical plant and WPCP, but did not detect the CP's.	- CPS may be more significant than water monitoring would indicate. - Non-Point sources contributing.	- Trout toxicity test cn effluents for Ontario side indicated one predominant source if acute toxicity. Daphnia and minow toxicity texts. U.S. effluents identified one predominant source of acute toxicity.	- No toxicity data from effluents were applied to the problem.	- Effluent chemical monitoring concurrent with in-stream biological monitoring. - Amendments made to effluent permits based on chemical and biological data from receiving waters.

These factors together often result in important but unavoidable data gaps. This point may be a strong reason for employing sensitive sub-lethal toxicity tests (e.g. reproductive, developmental, behavioral) directly on effluents at source, to ensure their relative safety to the environment.

Table 7 summarizes key elements of the selected case studies, which were selected on the basis of their location, importance, type of problem encountered and availability of data and other information.

In the Upper Saint John River study, the relationship between toxicity and BOD removal in the treated effluent was probably coincidental. The mill's primary objective was to reduce effluent BOD. In doing this, the combination of process changes and improved effluent treatment also resulted in a non-lethal effluent. The materials responsible for the effluent's toxicity, such as terpenes and phenolics, were not individually identified. Chemical water quality monitoring in the river showed an improvement in dissolved oxygen levels and biomonitoring of the effluent indicated a reduction in the loading of toxic materials to the river.

The Canagagigue Creek study was a detailed survey of a stream environment suspected of receiving high levels of contamination from both controlled and uncontrolled sources. By design, effluent biomonitoring did not play a significant role. There was infrequent toxicity or chemical testing of the effluents by the provincial agency and toxicity testing was limited to a standard fish test measuring lethality. Hence, the effects of the discharges on the stream were described through detailed ambient chemical and biological analyses (from transformation products to residues in leeches and fish). The primary materials of concern entered the stream as leachate from a waste site on the chemical plants' property, as well as from the treated effluent from the adjacent WPCP, an important finding that would have been missed if all work had been on the effluents.

In the lower Saint John River study, a specific biological monitor was tested in an area suspected of having industrial organic contamination. Leeches indicated the presence of chlorophenols, confirming an earlier result (27). The chlorophenols would have been undetected through routine water quality monitoring, as currently conducted in Canada, pointing out the importance of a biological monitor.

The Niagara River receives persistent pollutants from industrial effluents and landfill leachates. Biological monitoring of tissue levels and community structure of the river is being used to describe the problem and to monitor the efficacy of controls. Monitoring the toxicity of effluents with sensitive and practical tests seems to be playing a small role at present, an approach to the problem which could be questioned if the objective is to curtail point-source pollution rapidly.

Tobin Lake acts as a sink of contaminants from a range of industrial and non-point sources. Some of the industrial discharges (e.g. pulp mills, oil refineries) are operating under provindial licence and it is not known whether their effluents are in compliance with the chemical and toxicity limits in the federal regulations. Biological testing detected organic compounds in the sediments that caused mutagenic and developmental effects in selected test organisms. The bioavailability and toxicity of the compounds to endemic organisms is not yet established, nor have the sources of the compounds (agricultural, industrial, or air-borne) been identified. Thus far, end-of-pipe biological testing of effluents at locations upstream has not played a role in this study.

In the case of the smelter on the Columbia River, sampling of the effluents has been undertaken concurrently with ambient chemical and biological sampling. The concern with the effluents is the load of metals and volume of acid discharged to the river, a concern identified initially with the assistance of acute lethal fish toxicity tests. Permits are issued now to control the level of pH and metals in the effluents, and are based on water quality conditions in the receiving waters, not on the results of continued toxicity tests on the effluents themselves. The knowledge gained through the present study will provide the industry and government agencies with the information required to control effluent discharges under varied river flow and operating conditions. Water quality objectives may be developed in future to facilitate this process.

The case studies discussed above, and the experience of many practitioners in Europe and North America, show that biological tests applied to effluents at source and in receiving waters have an important role in water pollution control. The specific test or biological procedure chosen must be carefully selected to address the questions posed by each unique problem. Many effluents in Canada have been evaluated for toxicity with the acute rainbow trout test, which identifies highly noxious effluents, highly toxic components or characteristics of effluents, and improvements in effluent composition subjected to treatment. The trout test is used frequently in Canada; nevertheless, short-term, sensitive and predictive biological tests should now be applied more frequently to non-lethal effluents at source and in-situ. There is a greater need to improve and calibrate existing procedures (e.g. QSAR approaches, enzyme assays, egg-embryo-larval tests, in-situ bioaccumulation and histopathological approaches, and community responses) and to use them to resolve continued serious pollution than there is for new toxicity and ambient biomonitoring techniques.

The case studies also show that the sublethal effects of mixtures of toxic substances at low concentrations, particularly from effluents, require continued study. Known combined effects of major classes or types of toxicants are considered superficially in the setting of water quality objectives. Much more basic and applied knowledge is urgently required in this field.

There should be a continued effort to correlate effluent biomonitoring tests with those useful for monitoring and predicting contaminants effects in-situ. Effluent tests used in Canada are diverse and include many responses and organisms (2,24); but correlations with results of field monitoring are relatively scarce. Further work is essential in support of specific studies such as those discussed above. Such correlations are also needed for effluents entering saltwater bodies, a topic not addressed in this paper.

Above all else, problems of toxic substances in effluents must be addressed with both effluent and ambient biological tests using modern techniques. Many administrative decisions can and have been made from the results of existing and state-of-the-art biological tests. The key question: are we protecting each unique water body adequately?, can only be answered by the continued application of practical, realistic, sensitive, and predictive biological and chemical approaches backed by basic environmental science. Our case examples demonstrate the ingenuity, flexibility, cooperation, and commitment required to meet this challenge by both administrators and scientists.

ILLUSTRATIONS

Tables

Table 1 Historical characteristics of effluent data from the pulp and paper mill at Edmunston, New Brunswick.

Table 2 Acute toxicity of effluents entering Canagagigue Creek, Ontario (32).

Table 3 Disappearance rate constants (K_D, hr^{-1}) and halftimes ($T_{\frac{1}{2}}$, hr) for 2,4-DCP and 3,4-DCP, 1981.

Table 4 2,4,5-Trichlorophenol levels in Canagagigue Creek biota and water at site CN-5 on four occasions in 1981.

Table 5 Comparison of bacterial and nematode bioassays on fractions of Tobin Lake sediments (36).

Table 6 Some applications of end-of-pipe toxicity testing (W.R. Parker, personal communication).

Table 7 Evaluation of selected case studies.

Figures

Figure 1 Process for developing water quality objectives in Canada.

Figure 2 Application of water quality objectives to a water management area.

Figure 3 Monitoring program to determine compliance with water quality objectives in the Saint John River Basin (19).

Figure 4 Water sampling stations on the international waters of the Saint John River Basin.

Figure 5 Map of Canagagigue Creek study area (13).

Figure 6 Population distributions of some of the dominant benthic organisms from Canagagigue Creek in 1980.

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Biological Monitoring of the Marine Environment in Relation to the Disposal of Sewage Sludge

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INTRODUCTION

At the present time the United Kingdom disposes of about one third of its sewage sludge, approximately 10 million wet tonnes, to the sea at 13 licenced sites around the coast of the UK (fig. 1). The responsibility for monitoring and surveillance of the sites lies with the Ministry of Agriculture Fisheries and Food (MAFF) or the appropriate Governmental Agency in Scotland, Wales and Northern Ireland. The monitoring and surveillance involves measurement of chemical contamination in the different levels of the ecosystem and these are related to the loading to the environment of the contaminants involved in the waste disposal. In addition to this species numbers and diversity in and around the disposal area are determined from benthic surveys. Pearson and Blackstock(1982) in studying a sewage disposal ground have identified changes in the macro benthic fauna which can be related to changing sediment conditions brought about by organic enrichment. They found high numbers, high biomass and low species diversity at the centre of the ground and low numbers, low biomass and high species diversity in the areas beyond the influence of the sludge input. This pattern is analogous to the pattern observed in limnology as a result of eutrophication.

By the time that a detectable shift in species has been observed it is probable that significant damage will have been inflicted on the environment. It is preferable to be able to detect the initial changes in physiology and cell chemistry that eventually lead to a decline in the species in the area. In order to be able to monitor these more subtle changes it is necessary to choose a suitable target organism by which to measure the changes. A suitable animal for use in sewage disposal grounds is the mussel Mytilus edulis for which there is a considerable background of basic physiological information. The technique using the mussel involves measurements of basic physiological processes which allows the energy buget to be calculated and also examination of the tissues of the digestive gland. This latter technique can yield information regarding intracellular detoxification processes and also changes in cell structure.

PHYSIOLOGICAL RESPONSE TO STRESS

Changes in the energy budget of the test organism may be used to indicate a state of physiological stress, this may be due to purely natural phenomena such as gamete production or it may be due to an external influence such as the presence of harmful materials in the water following sludge disposal. The measurement is termed Scope

For Growth (SFG) and the technique is described in detail by Widdows(1982).Mussels previously exposed to the conditions in the study area are placed in controlled conditions in the laboratory and measurements made of their feeding, assimilation, respiration and excretion rates. These rates are converted to energy equivalents and the energy budget for the animal is calculated and then compared with the budgets of similar animals that have been exposed at an unpolluted site. A significantly lower value of SFG in the test animals indicates that the population is in a stressed condition.

SUB-CELLULAR RESPONSES TO STRESS

When an organism is exposed to toxic materials a defence mechanism of detoxification pathways may be induced in the tissues most closely associated with the intake of the material. In recent years it has become possible to look at sections of tissue taken from animals and by using histochemical techniques to determine the condition of the cells and organelles. The Water Research Centre in collaboration with the Institute for Marine Environmental Research are currently investigating a number of such techniques with a view to their use as monitoring techniques. These will allow the effects of metals and organic xenobiotics to be detected. All the techniques involve exposure of the test organisms for a period of time, usually 60 days, to allow them to become equilibrated with the new environmental conditions. The digestive glands of a number of animals exposed at each site are removed and fixed by freezing in liquid nitrogen. This prevents deterioration of the cells and holds the biochemical pathways as they were at the time of collection. In the laboratory the tissue is cut into thin sections and processed appropriately to demonstrate the presence or absence of the inducible biochemical pathways.

The presence of an active metal detoxification system is shown by staining the metal-protein complex, metallothionein, using the technique described by Shikata et al(1974), each section is scored +ve or -ve to indicate the presence or absence of the complex.

Many organic xenobiotics, including aromatic hydrocarbons and pesticides are metabolized by mixed function oxygenases in the microsomes, the proliferation of which is stimulated by many foreign compounds. The activity of the system can be detected by a colour change in stained tissue.

The lysosomes in the cells of the digestive gland of the mussel form part of the intracellular digestive system and one of the properties of the membrane bounding the lysosomes is that the stability may be reduced under conditions of physiological stress, the reduced stability allows the release of previously bound enzymes into the cell. The stress can be induced as a result of exposure to a wide range of both organic and inorganic pollutants and so the technique can be used as a broad band monitor. The method involves the deliberate destabilization of the membrane in sections of the digestive gland of exposed mussels. Sections are treated for different time periods and then stained, a short period of treatment resulting in +ve staining indicating greater original membrane instability and hence higher stress. Both of the techniques are discussed in detail by Moore(1980).

RESULTS OF TRIALS IN A DISPOSAL GROUND

The techniques have been used to monitor a sewage disposal ground of about 5 square kilometres in water to a depth of 50m. in the sea off the south coast of the UK. Mussels collected from a nearby unpolluted area were exposed for 60 days along a transect with the disposal ground at its centre (Fig 2). They were held in cages supported by an anchored buoy system 4m below the surface and 4m above the sea bed. Sewage sludge was regularly discharged in the area at a rate of 300 cubic metres per day. Following the exposure the mussels were recovered and the physiological and cellular effects measured.The results of the measurements of SFG and sub cellular changes are shown in Fig 3.

SFG was highest at Site 1, furthest away from the disposal ground for both the upper and lower cages and lowest in the upper cage at Site 5. The upper cages generally had a lower SFG than did the lower ones. The animals in the upper cages had all increased in wieght with a maximum at Site 6, the centre of the disposal ground while those in the lower cages had not increases as much as the native population.

Lysosomal stability showed that all the upper animals were stressed, particularly in the vicinity of the disposal ground (Sites 5 and 6) and least so at Sites 1 and 7. None of the upper animals were stressed as much as the ones in the lower cages but Site 6 was the most stressed of the lower cages. Both of these techniques are measures of general response to stress and it requires further information from the more specific tests to identify the type of contaminant responsible for the condition of the test organism.

The Shikata test for metallothionein shows that of the animals in the upper cages only those at Site 1 were stressed by metals while those in he lower cages recorded +ve at all sites except Site 3, the highest responses being at Sites 5 and 6. The high value at Site 1 may be attributable to the known high levels of metals in the sediments of the adjacent estuary. The pattern of metallothionein in the cells is also found in the measurements of increased metal concentrations in the soft tissue.

The results for the organic xenobiotic indicator show that the animals in the upper cages were significantly more stressed than those in the lower cages, this could be caused by the presence of an organic xenobiotic in the sludge or perhaps the natural organic nature of the sludge itself.

It can be postulated that the animals most exposed to the sludge, those in the upper cages at Sites 5 and 6, used it as food source and increased in weight, they were not stressed by the metals in the sludge and did not accumulate any of te metals in their tissue. The evidence of stress points to either the organic material of the sludge itself or possibly an organic xenobiotic present in the sludge. It is possible that the animals in the lower cages were exposed to a denser metal carrying fraction of the sludge and that this accounts for their apparent response to metal contamination.

FUTURE EVALUATION OF THE TECHNIQUES

The initial trial carried out using these techniques indicates that they may be suitable for routine monitoring of disposal grounds and for comparing effects of discharging different types of waste materials and estuarine inputs . The techniques will be tried out on a number of different areas of the UK coastal waters and estuaries with intensive laboratory investigations to look at the dose response curve with a view to determining which constituentsof sludge trigger particular responses im mussels. These investigations intend to provide a perspeotive on the environmental input of sludge disposal compared with other inputs of contaminants to the sea and to contribute towards a scientific basis for sludge disposal policy.

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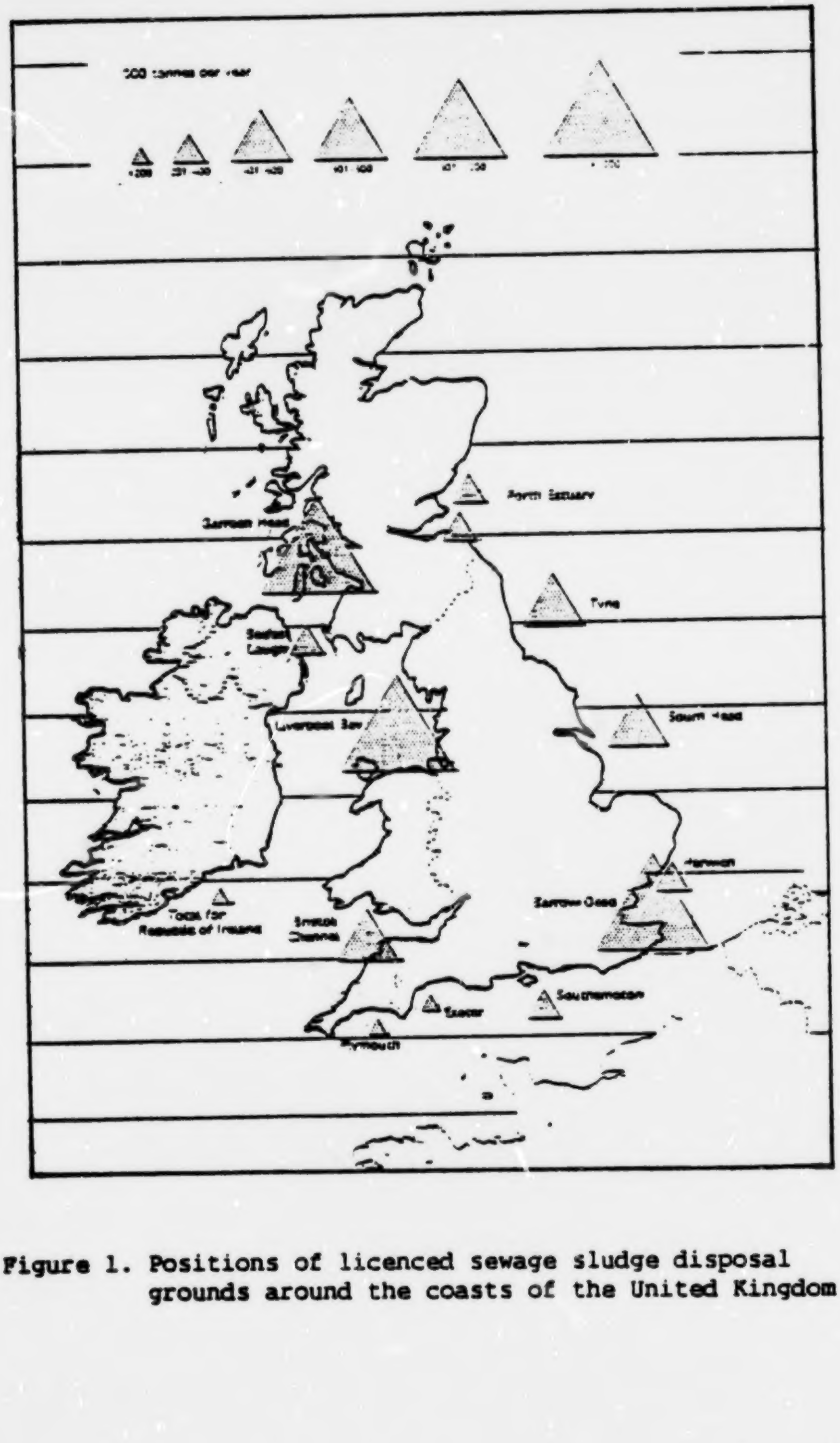
'000 tonnes per year
Forth Estuary
Garroch Head
Tyne
Belfast Lough
Liverpool Bay
Spurn Head
Harwich
Barrow Deep
Tocks for Republic of Ireland
Bristol Channel
Southampton
Exeter
Plymouth

Figure 1. Positions of licenced sewage sludge disposal grounds around the coasts of the United Kingdom

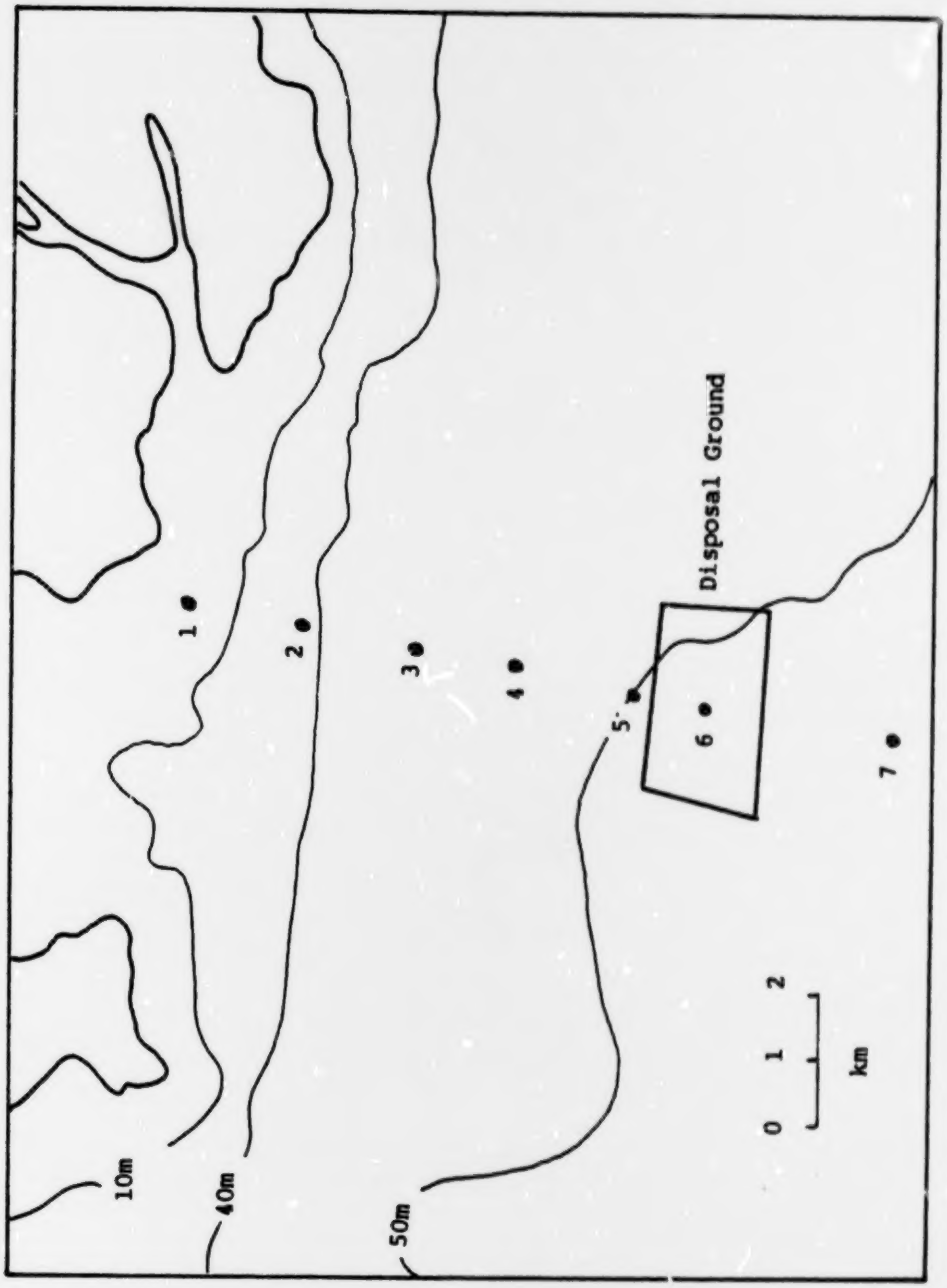
10m
40m
50m
1
2
3
4
5
6
7
Disposal Ground
0 1 2
km

Figure 2. Positions of trial monitoring sites in the disposal ground

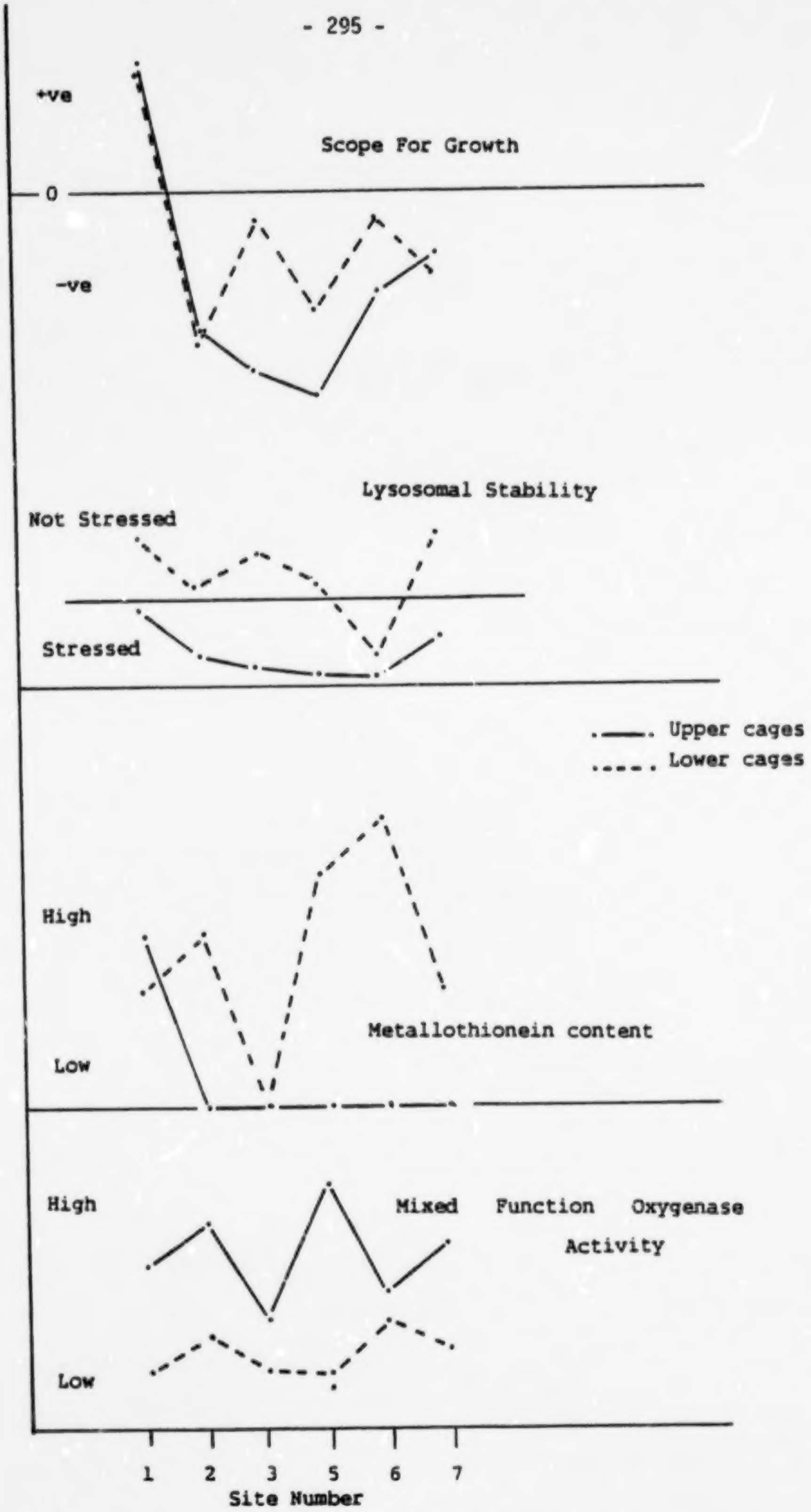

Figure 3. Results of physiological and cytochemical tests on Mussels exposed on the trial transect

BIOLOGICAL TESTING OF EFFLUENTS AND ASSOCIATED RECEIVING WATERS - UK EXPERIENCE.

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Background

Historically, discharges controlled under the Rivers (Prevention of Pollution) Acts of 1951 and 1961 have included the usual numerical consent (licence) conditions relating to the chemical composition of the effluent. Some consents have also included an all embracing statement that the discharge should not be injurious to fish, and the associated ecology of the river.

Legal advice on the general principle of including such a condition was that it was not enforceable and that consent conditions should relate to the discharge not the receiving water although provisions were contained in the Salmon and Freshwater Fisheries Acts of 1923 and 1975 that made it a prosecutable offence to harm or damage freshwater fish, their spawn and spawning grounds and their food.

In the UK the purpose of controlling effluents (both sewage and industrial) discharged to the aquatic environment has been to improve and to protect the use that is made of the water, be it for abstraction for public supply, support of fisheries or general amenity. With the increasing number and complexity of chemicals used in industry and with substantial amounts of trade effluent being discharged to the sewer for treatment in admixture with domestic sewage, increasing attention has focussed on a standardised test to determine the acceptability of an treated effluent when discharged to the receiving water. A variety of test methods had been developed in the 1960s and in 1968 the then Ministry of Housing and Local Government (MHLG - the predecessor of the present Dept. of the Environment) set up a Technical Committee to consider two such tests, namely a flow-through test developed by the Ministry of Agriculture Food and Fisheries (MAFF) in which the diluted effluent was continuously renewed over the test period of 48 hours and in order to reduce the amount of sample required, a small tropical fish was used - the harlequin fish (Rasbora heteromorpha). The other test the committee considered was a simpler one developed by the former Trent River Authority (TRA) in which, by using a static open test, no attempt was made to prevent loss of volatile toxic components of the effluent, and the fish used was brown trout (Salmo trutta). The Technical Committee in its report of August 1969(1) recommended a standardised fish toxicity test procedure based on the TRA method of 48 hour duration using ten rainbow trout (Salmo gairdneri) between 60 and 150 mm in length. Two alternative dilution waters of 25 and 250 mg/l hardness (as $CaCO_3$) were specified, the one most appropriate to the receiving water being chosen for the test.

A comparison of the two types of test had been carried out for the Technical Committee and had demonstrated the greater sensitivity of the enclosed continuous flow test of MAFF particularly when effluents contained volatile or readily degradable substances but the TRA test in a standardised form was considered more suitable for routine use. It had been found using ten different effluent samples that on average the concentration of effluent sample which gave a 50 percent mortality in the static TRA was about twice that in the continuous flow MAFF test. Emphasis was laid on the fact that the tests were empirical and that such tests should be used with discretion.

A considerable amount of interest had centred on the early work done on simple mixtures of toxic substances, mainly metals and some of the common components of the treated effluent from, for example, the gas industry. Much of this work was carried out at the laboratories of the former Water Pollution Research Laboratory at Stevenage (now part of the Water Research Centre) and has recently been referred to in the present context by Lloyd(2). These studies include predictions of the toxicity to fish of sewage effluents containing residues of trade wastes, based on chemical analysis. The apparent success of this approach may have inhibited the use of toxicity tests to control discharges.

So far as is known the MHLG standard fish toxicity has been very little used as a consent condition. Price reported(3) in 1981 that to his knowledge there were only two discharges controlled in that manner, both in the Severn-Trent Water Authority area, one being a sewage works effluent containing wastes from a chemical manufacturers and the other a discharge of leachate from a waste disposal site.

Water Authorities do, however, make use of fish toxicity tests in an informal manner, either based on the MHLG test or some suitable alternative test to ascertain the acceptability of an effluent and its effect in a diluted form, in the receiving water.

Indeed a considerable amount of work has been undertaken by the Department of the Environment Standing Committee of Analysts to standardise some of these further acute toxicity tests. Detailed test procedures for carrying out bioassays in a variety of waters and effluents have been published(4) and cover the use of rainbow trout (*Salmo gairdneri*), and two crustaceans *Daphnia magna* Straus and the brown shrimp (*Crangon crangon*).

It should perhaps be noted that it is necessary for any laboratory where it is proposed to carry out fish toxicity tests to be registered with the Home Office under the Cruelty to Animals Act of 1876, operators require a licence and certificate under the Act and premises are inspected.

It is also relevant to point out that at present the disclosure of information relating to effluents is restricted under Section 12 of the Rivers (Prevention of Pollution) Act 1961 and even when registers are open to the public under Section 41 of the Control of Pollution Act, 1974, details pertaining to an effluent, other than those prescribed in Regulations, will still be restricted.

Review of present day use of biological testing and monitoring by some of the water authorities

a. Discharges

The Southern Water Authority(5) have found in studying the effects of the final treated effluent discharged from the petrochemical and waste treatment industries to coastal and estuarial waters that rainbow trout (200-300mm), could eventually be acclimatised to brackish or sea-water, but presented practical problems of being too large and aggressive for use in a 20 litre static test, and mortalities were high during acclimation. However they found that dover sole (Solea solea) mean length 80-100mm were very tolerent to the wide range of salinity variations that are found in an estuary and that occur in test tanks when low salinity effluents are added to sea water. In static laboratory tests the LC 50 tests are carried out using 10 fish in the test water prepared from sea water diluted with fresh water to a salinity of 30 $^{0}/00$. The fish are kept in the diluted sea water stock tanks for 7 days then introduced to the test water of the dilute sea water and effluent, usually at concentrations 0, 0.1, 0.4, 1.6, 6.2, and 25 percent of effluent. After 48 hours the test water is replaced and the test continued for a further 48 hours. Tests are conducted at 15 ± 2°C with a dissolved oxygen greater than 80% of the saturation value. The LC 50 for dover sole had been found to be of the same order as that given in the literature for other fish, such as flounders and trout, when using for example copper or phenol was present in the water.

The Yorkshire Water Authority(6) have indicated that although they have no consents that include bioassays they have informal agreements, such as with one toxic waste disposal site owner that leachate and surface water can only be discharged from a lagoon to a nearby stream if shown to be non toxic to Daphnia in a 24 hr LC_{50} test.

The Authority have also operated, on a continuous flow through basis for periods of up to 10 weeks, laboratory scale tests in which domestic sewage and complex chemical wastes, such as from pesticide manufacture and tankered to a major sewage treatment works, are evaluated in order to obtain an environmental impact assessment. The procedure involves the treatment of the sewage and trade effluent in six 3 litre WRC porous pot activated sludge units (3 control, 3 treatment) and the final effluents from each set of three pots are collected together and passed through a fish tank (working volume 35 litres) containing juvenile roach (*Leuciscus rutilus*) in a long term toxicity test. The domestic and trade effluent are fed at appropriate rates to the activated sludges units and as the trade effluent composition is variable the approach allows any intermittent toxicity to fish to be observed and could enable the system to screen out any process wastes that would be detrimental to the river.

In common with most of the Water Authorities in England and Wales, the Anglian Water Authority carries out routine 48 and 96 hour static tests with the aim of protecting flora and fauna downstream of a discharge. Often there is a lack of toxicological information to quantify the effect of an effluent by chemical data and/or because of the analytical difficulties in identifying and measuring quantitatively all the active isomers and products of a particular chemical process. Recently the Authority(7) in conjunction with MAFF have developed a test based on the brown shrimp (*Crangon crangon*) and incorporated it as a consent condition for the discharge of trade effluent, (2045 m^3/d) from the manufacturer of agrochemicals to an estuary which has a 150 x 10^3 m^3/d low freshwater flow. In the test two test tanks of 10 litre capacity containing 2 litres of the effluent sample diluted with 8 litres of sea water (30 to 35‰ salinity) at pH 7.5 to 8.0 and at 15± 1°C are used, together with a control tank of dilution water only. The brown shrimp (40 to 60mm length excluding length of any antenna) are acclimatised to the test conditions in dilution water for 2 to 4 days prior to the test. At the commencement of the test 20 creatures are hand netted from the acclimatisation tanks(s) and placed in each of the two tests tanks and the control tank. The tanks are continuously aerated to maintain a dissolved oxygen air saturated value of not less than 80% and the diluted samples and control water are changed each 24 hours during the 96 hour test. The sample of effluent is deemed to fail to satisfy the conditions of the consent if the cumulative percentage mortality in each of the tanks exceeds 50%. One of the substances the treated effluent can contain is the pesticide chlorpyrifos which has a 96 hour LC 50 to brown shrimp of 0.28 ug/l; the chemical condition in the consent for chlorpyrifos is that it should not exceed 3 ug/l.

Apart from supporting fish life, river water is extensively used in irrigating crops both in the field and in glasshouses. In 1973 a serious malformation of young tomato plants occurred among commercial growers in Essex. It was demonstrated that the effects were those produced by 2,3,6 trichorobenzoic acid (TBA) - a substance present in the treated trade effluent from the manufacture of pesticides that was being discharged in the Great Ouse catchment from which water was transferred to the Essex rivers (8 and 9). Tomato plants are extremely sensitive to 2,3,6 TBA and in laboratory tests watering the plants with a solution containing as little as 0.5 ug/l of the substance produced detectable effects. Whilst a stringent chemical condition of 20 ug/l of 2,3,6 TBA was included in the consent and the manufacturer took extensive and expensive measures to reduce the discharge of TBA it was recognised that the chemical composition of the effluent could be variable and complex and that a form of bioassay test was necessary to prevent a reoccurrence of a similar incidence. A condition incorporating such a test using tomato plants has been described by Price (3). Further developments and extension to other test plants including barley, cucumber, pea and radish are outlined in the Appendix.

b. Associated receiving waters

One of the significant advantages of biological testing is that it can reveal episodic events that can be missed by spot sampling or long term chronic toxicity tests. Such an event can arise from heavy rainfall and the operation of storm sewers quite apart from the "one off" pollution incident, be it an accident or one of those "Saturday night" discharges! All water authorities are only too well aware of the pollution arising from the accidental agricultural discharge of silage liquor or manure slurry and frequently the first sign of pollution is the sighting of dead fish in the river and this can be too late to protect other river uses.

Much attention has been given by the Water Research Centre to developing a suitable early warning system to protect, in particular, water treatment plants. There are some 150 such plants in the UK abstracting water for potable use from rivers.

About half of these do not have intermediate bankside storage facilities to act as a buffer against low flow or a pollution incident. In 1983 there were about 12,000 reported river pollution incidents in the catchments of the English and Welsh Water Authorities and typically there will have been perhaps 100 closures of water intakes as a result of these incidents.

Not all the intakes have on line monitoring facilities and those that do will probably have monitors mainly for ammonia, suspended solids and dissolved oxygen. There are a few sites which use a biological monitor, usually fish and in the simplest form the monitor consists of a tank containing golden orfe, (Leuciscus idus) gold fish, (Carassius aupatus) guppy (Labister reticulatus) or small rainbow trout, (Salmo gairdneri). The tanks are checked at regular intervals and erratic behaviour or death used as an indication that the water contains something distressing to the fish and by implication it might be unacceptable to the human consumer. The limit of detection of this technique is obviously high, up to 2 orders of magnitude higher than those obtainable by reference to the ventilation frequency of the fish.

Over the last eight years the Water Research Centre has developed a number of automated fish monitors based on more subtle changes in physiology. The Mark III monitor(10) makes use of the change in gill ventilation (breathing frequency) of rainbow trout and is capable of raising an alarm within 15 minutes of the arrival of certain pollutants. Two demonstration units incorporating the latest monitor of the commercially available equipment are currently being evaluated by the Water Authorities.

Such a monitor need not of course be confined to sampling river water prior to abstraction for treatment; it could also be used to monitor the quality of effluents prior to discharges, see table 1.

Table 1 Response of Mk III Fish Monitor

Pollutant	Limit of detection mg/l	Time to first significant change in ventilation frequencies of fish (minutes)	96hr LC_{50} mg/l
ammonia	0.03	36	0.16 - 0.49
phenol	3.75	12	9.3
paraquat	0.8	12	
γ HCH	0.003	9	
petrol	2.0	49	
diesel	10.0	22	
gas oil	2.0	47	

An alternative to the use of fish monitors was developed some years ago by Holland and Green(11), and field trials in the Severn-Trent Water Authority were reported by Stroud and Jones(12). Basically the "Gross Pollution Detector"(GPD) consists of a specific ion electrode for ammonia that automatically detects the inhibition, by pollutants, of the nitrification of ammonia in a biological filtration column. Holland and Green had demonstrated the response of the nitrifying column to the presence of a number of pollutants and had obtained. toxicity threshold concentrations for various pollutants - table II

Table II

Pollutant	Toxicity threshold concentration mg/l
cyanide	0.04
allyl thiourea	0.1
endosulphan	saturated (approx 0.1)
diquat dibromide	0.1
phenol	1.5
cadmium	2.0
copper	4.0
paraquat dichloride	4.0
chromium	5.0
γ BHC	saturated
malathion	750

The Water Authority have installed a number of the monitors at sites particularly where treated sewage effluent can be a major component of the river and the river is abstracted for potable supply. The mode of operation is to add 2mg/l NH_3 as ammonium chloride to river water, which typically has an ammonia level of about 0.3mg/l, that is continuously being withdrawn from the river and fed to the top of a small biological nitrification column. Contact time is adjusted to about 30 seconds and the ammonia content of the outlet is monitored with an ammonia probe. Normally about 90% of the ammonia is removed leaving a residual of 0.2mg/l NH_3. The presence of a pollutant can inhibit nitrification hence the ammonia content of the outlet water from the filter rises. The manner of the increase and comparison with the 4 hourly standardisation check on the inlet to the column, together with other normal monitoring information often assists in the detection of the likely source of pollution.

One such installation is at Stoneleigh on the river Sowe, downstream of Coventry and its Finham sewage treatment works (average daily flow 116Ml/d) where the treated effluent constitutes some 50% of the flow in the Sowe, the remainder of the flow being mainly urban run-off. At another site, Draycott, on the River Derwent, the monitor has served to warn and confirm when pollution incidents have arisen due to discharge of refrigerant from a factory some 4km upstream, when an agricultural pollution incident occurred and when quality of the treated sewage effluent (average daily flow 97Ml/d) deteriorated from the town of Derby. Downstream of the monitoring station is Church Wilne, a major intake for public supply. The GPD is linked by VDU with the water intake which can be shut down on detection of pollution and an automatic sampler commences taking samples at 15 minute intervals in order that the pollutant can be identified by conventional chemical methods.

At present the Authority see the GPD as another tool in assisting in monitoring the quality of effluents from many sources, and as a water intake protection device(13). It is recognised that there are limitations to its sensitivity,but when used in conjunction with other chemical monitors useful and relatively cheap information can be supplied. The main advantage lies in its ability to detect gross pollution of a much wider range of substances than many other detectors.

In the international scene biological monitoring and the testing of effluents is receiving recognition. Thus in the EEC Directive concerning the discharges from the titanium dioxide industry and their impact on the surrounding environment, monitoring of the flora and fauna has to be carried out(14). In the UK the two factories situated on the south bank of the Humber estuary have in conjunction with the Anglian Water Authority carried out extensive monitoring and observations of the ecology within the area of the discharge and made comparison with that in an area outside the area of possible influence. In the Directive concerned with the discharge of mercury from the chlor alkali electrolysis industry(15) the concentration of mercury in a representative sample of fish flesh chosen as an indicator must not exceed 0.3mg/kg wet flesh and in the Cadmium Directive(16) the concentration of cadmium in sediments and/or shellfish, (if possible the species Mytilus edulis), must not increase significantly with time.

In UK waters some 10 million tonnes per year of wet sludge mainly from sewage treatment works are disposed of to 13 coastal sites. The disposal of wastes to sea is controlled by the Dumping at Sea Act 1974 which requires the authorities (MAFF), when granting a licence, to have regard to the need to protect the marine environment and its living resources from any adverse consequencies of waste disposal. There are international pressures within the EEC and the Oslo and London

Conventions which are seeking to further restrict the disposal of sludge to the sea. In order to demonstrate that the environmental impact is acceptable and that the quality of the sludge and the manner of disposal is in accord with responsible management and good practice, MAFF carry out extensive chemical and biolgoical tests before a licence is granted, and they subsequently monitor the hydrology, sedimentology, chemistry, benthic ecology and at times the microbiology of the dumping grounds(17). More recently, MAFF have developed the oyster embryo bioassay to measure the biological water quality at dumping grounds and WRC in conjunction with the Institute for Marine Environmental Research (IMER) are investigating the utility of other sensitive bioassays for this purpose. Details of this work are reported in the paper by Johnson, Lack and Davis of the Water Research Centre presented at this Workshop(18). Other tests carried out in the UK on wastes and chemicals disposed of at sea have been recently described by Lloyd(19).

Summary

The policy adopted by the UK of identifying the present and future uses of the water in the environment and hence the quality necessary to sustain that use (the EQO/EQS philosophy) has meant that considerable attention has been paid to the effect that discharges might have on the natural ecology of the receiving water.

Examination of the literature pertaining to UK experience and discussion with officers of the water authorities indicates there has been an increasing use made of biological testing of effluents in order to make some qualitative assessment of the impact of discharges to the aquatic environment. Water authorities and their predecessors have used some form of fish test (static/flow through, acute toxicity/long term toxicity) as a means of pre-assessing and monitoring the effect of various substances in effluents. The early work was mainly directed towards single substances and simple mixtures and the correlation of the observed effect, in the laboratory and the field, with the chemical concentration. Initially much of this work was focussed on inland waters but more recently has extended to marine waters.

In addition to testing effluents there has been a long experience of biological monitoring of the receiving waters as authorities responsible for the preserving of water quality of the rivers have also sought to "have regard to the desirability of preserving natural beauty, of conserving flora, fauna and geological or physiographical features" [The Water Resources Act 1963 s. 101]. This is additional to the responsibility they have "for maintaining or restoring the wholesomeness of the rivers and other inland or coastal waters" [Rivers (Prevention of Pollution) Act 1951]. The Water Authorities also will shortly have, as a result of the implementation of Control of Pollution Act 1974 s.46, (COPA,) all the necessary powers they may require to remedy or forestall pollution of water that may be injurious to the fauna or flora of a stream and where necessary to take appropriate action and recover the costs of such operations from the persons who made the discharges. Under the wider provisions of consents that may be granted under COPA it is open to the Water Authority to require the discharger to carry out some form of biological monitoring of the effluent being discharged. There are of course, increasing international pressures for the inclusion of various biological monitoring tests both of the effluent and the associated receiving waters as seen in the Directives concerned with discharges containing cadmium and mercury and the waste from the titanium dioxide industry. In some cases a "standstill" approach has been adopted in that the toxic metal content of shell fish and sediments has not to increase, it being argued that fish provide, in such circumstances a good and effective biological monitor.

The importance of toxicity testing of effluents and the biological monitoring of effluents is recognised in the publication of test methods by the Standing Committee of Analysts in their series of "Methods for the Examination of Waters and Associated Materials" and it now appears that with a standardised test method a suitable condition could be included in a consent for the discharge of an effluent.

The biological testing of effluents has not been confined to acute toxicity tests on fish; both long term toxicity tests and bioaccumulation assays are carried out to study the effects of the effluent in the receiving waters.

Over the years MAFF have indirectly made an important contribution by their work of monitoring the amounts of toxic metals and organo chlorine compounds in marine and shellfish flesh for human consumption, that has served as an indicator of their presence and concentration trend with time. Physiological tests involving breathing or ventilation, heart beat and avoidance also play an important part. The inhibitory action of some substances on nitrifying bacteria can also be used to good effect and whilst these are not acute toxicity tests they do provide useful early warnings or indications of potentially polluting situations arising and when used in conjunction with other chemical tests they help complete the effectiveness of control of effluents of a variable complex nature particularly where episodic events can occur. Phytotoxicity and the malformation of certain plant growths also can provide the basis of suitable methods for establishing control of effluents.

At present in the UK the application of these various techniques appears to be non routine and irregular and a somewhat fragmented picture has emerged. The main industrial activities to which some form of biological testing has been applied are those concerned with the production of titanium dioxide, pesticides and herbicides, and oil and petrochemicals. The latter industry has been particularly active in applying some of the techniques to its effluents and the results have been reviewed by the European oil companies international study group for the conservation of clean air and water (CONCAWE) who have also held a workshop on the subject(20).

It is expected that the future use of these techniques in the UK will increase, for anticipatory purposes before a discharge is authorised, for the control and monitoring of the effluent and its impact on the aquatic environment, and as a means of providing an early warning in order to protect water uses and the general preservation of the environment.

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PLANT BIOASSAY TEST FOR UNKNOWN HERBICIDE INCLUDING T.B.A. SPECIFIC TEST

1. Procedures

The Procedures in the test will be carried out under the supervision of an independent graduate biologist nominated by the Dean of Natural Sciences of Hatfield Polytechnic, or nominated by such other person as may be agreed upon by the Authority and the Company, and the opinion of such graduate biologist as to whether or not the test solution has caused the effects mentioned in Section 7 of this schedule shall be evidence in the event of the Company being prosecuted for a contravention of the terms of the consent.

2. Test Plants

The test plants and the variety shall be

(a)	Tomato	Eurocross B.B. or Money Maker
(b)	Pea	Feltham First
(c)	Radish	French Breakfast
(d)	Cucumber	Butcher's Disease Resisting
(e)	Barley	Maris Otter

and all free of any discernible disease.

3. Cultivation of Test Plants

The test plants shall be cultivated as follows:-

(a) Sowing dates

Seeds will be sown as follows
Barley - 7 days before scheduled test start
Cucumber - 21 days before scheduled test start
Pea - 10 days before scheduled test start
Radish - 14 days before scheduled test start
Tomato - 21 days before scheduled test start

(b) Potting Compost

All plants to be germinated and grown in John Innes No.2 compost.

3. (c) Sowing procedure and test plant selection

Tomato, Radish and Cucumber.

For each variety seeds are to be sown in excess numbers in seed trays by scattering them over the soil surface and then covering them with 1 - 2 mm of sifted compost. Distilled water only is to be used for watering. Once the cotyldons are fully expanded 20 Radish seedlings and thirty seedlings each of Cucumber and Tomato are to be selected and transferred singly to 70 - 80 mm plastic pots, one seedling per pot. These are the plants to be used in the test.

Pea and Barley.

An excess number of Pea and Barley seeds are to be sown into 70 - 80 mm diameter plastic pots, one seed per pot, and then covered with a 1 - 2 mm layer of sifted compost. Distilled water only is to be used for watering. When they are 2.5 cm high twenty Pea and thirty Barley seedlings are to be selected. These are the plants to be used in the test.

(d) Cultural Conditions

Barley, cucumber, pea, radish and tomato seeds to be held in darkness at 22 ± 3°C until germination has occurred. Thereafter the seedlings to be placed under artificial light provided by warm white or daylight flourescent tubes giving an intensity of 7000 lux ± 2000 lux at soil level on a daily cycle of 16 hours light and 8 hours darkness.

(e) Transfer to the Glasshouse for Testing

Three days prior to the commencement of the test (when the cucumber, radish and tomato are at the first two true leaf stage, the first true leaf of barley is 30 - 40 mm high and the pea shoots are 20 - 30 mm high) the test plants are to be transferred to the glasshouse to acclimatise.

The following environmental conditions to be maintained throughout the test period:

Glasshouse temperature to be as close as possible to 22°C and a record kept of daily maximum and minimum values. The illumination cycle to be by natural light supplemented by artificial light (warm white or daylight flourescent tubes) of intensity 7000 lux ± 2000 lux for a period of 16 hours followed by a period of 8 hours darkness (provided it shall not be necessary to exclude natural daylight at the time of year when it exceeds 16 hours a day).

Whilst every effort should be made to keep as closely as possible to the specified environmental conditions some fluctuations will, from time to time, be inevitable. However, since evidence of phytotoxicity is based on a comparison with the control plants which have also been held in the same conditions such fluctuations as may occur cannot be held to influence the validity of the test.

4. Collection and storage of samples and test solutions

The sample of effluent to be tested to be collected in a glass container after the same has been cleaned with detergent, rinsed thoroughly with distilled water and then with some effluent. If the sample cannot be applied to the plants within 24 hours then it must be refrigerated to prevent anaerobiosis. The test solution to be stored in a refrigerator until application to the plants has been completed, but not to be applied to any plants until its temperature has risen to the ambient temperature.

5. The Test and Spiked solutions

The test solution shall be 1 part of neat effluent diluted with 14 parts of distilled water.

The spiked solution shall be neat effluent to which has been added sufficient 2:3:6 Trichlorobenzoic acid (T.B.A.) to give a concentration of 0.4 mg/l (assuming T.B.A. concentration to be zero in the neat effluent).

6. Test Procedure

The test to be carried out in a glasshouse and the temperature and illumination therein to be maintained as aforesaid. The distilled water and the test solution applied to the foliage of the plants to contain Tween 20' in a concentration of 0.1 percent. The control and test plants are to be treated in the same manner, one with distilled water and the other with the test solution (and spiked sample in the case of the tomato plants). Replicates of all treatments within each species to be randomised to eliminate any variation in growth response due to differences in conditions over the area of the glasshouse bench. The test shall be conducted as follows:

For Radish, Cucumber, Pea and Barley each species to be indifferently divided in to two equal sets, one set are control plants and the other are treatment plants.

For Tomato the plants are to be indifferently divided into three equal sets, one set are control plants, one set are treatment plants and the third set are spiked plants.

On the first, third and seventh days the control plants shall each receive 20 mls of distilled water, the treatment plants 20 mls each of test solution and the spiked tomato plants 20 mls each of the spiked solution. All applications to be at soil level. At the same time a quantity of distilled water shall be applied to the foliage of the control plants, a quantity of test solution shall be applied to the foliage of the treatment plants and a quantity of spiked solution shall be applied to the foliage of the spiked tomato plants by means of a fine horticultural spray sufficient to give maximum leaf coverage as evenly as possible without run off.

If it is deemed necessary to water either the control, treatment or spiked plants at any other time during the first seven days then distilled water shall be applied to all three sets of plants in the same quantity and manner.

After the seventh day all plants are to remain in the glasshouse for a further period of two weeks and watered as necessary with distilled water.

7. Phytotoxic examination

The treatment, control and spiked plants shall be examined and compared at the end of the second and third weeks, and any malformation or growth modification shall be recorded.

The test solution shall be deemed to have failed the test if the effects mentioned in a (i) - (vi) below are identifiable in more than one fifth of the treatment plants, provided that no similar abnormality is identified in more than two control palnts in which event the test will be null and void.

In addition, the three sets of tomato plants shall be compared against the attached photographs 1 - 10, these show tomato plants which have been exposed to various concentrations of 2 - 3 - 6 T.B.A. This test is only valid if the spiked plants exhibit similar characteristics to the photograph of the plant exposed to a similar T.B.A. concentration.

The test solution shall be deemed to have failed the test if abnormalities depicted in the photographs above number 2 are found to occur in two or more of the treatment plants, provided the control plants are all normal.

Furthermore, at the end of the third week the treatment and control plants shall be subjected to quantitative assessment as in b (i) - (iii) below. For each species the data shall be compared for treatment and control plants and any significant differences assessed by analysing for variance and significance at $P = 0.05$. This will derive a value for P (probability of a treatment being significantly different from the control). The test solution shall be deemed to have failed the test if P is 0.05 or less for one or more species.

a) Visual Assessment

At the end of the experimental period the treated plants will be examined for symptoms of phytotoxicity by comparison with the control plants. For the purpose of this test phytotoxicity effects are classified as:-

(i) Reduction in stem height of greater than 20%.

(ii) Leaf distortion - abnormal growth or a reduction in leaf area.

(iii) Chlorisis.

(iv) Leaf scorch or necrotic lesions not attributable to disease.

(v) Reduction in radish size.

(vi) Leaf or stem epinasty.

b) Quantitative Assessment

(i) Stem height of Cucumber, Pea and Tomato.

Stem height is defined as 'the height of the apical bud above the soil when the plant is fully extended, measured to the nearest 5 mm' Each plant to be identified and its individual height recorded at the end of the third week.

7. b) Quantitative Assessment

(ii) Leaf height of Barley.

Leaf height is defined as 'the height of the tip of the leaf above the soil when the plant is fully extended, measured to the nearest 5 mm' Each plant to be identified and its individual height recorded at the end of the third week.

(iii) Radish weight.

Each radish to be topped and tailed in a similar way as for eating and the individual weights recorded.

SOME EXPERIENCE FROM COMBINED BIOLOGICAL AND CHEMICAL TESTING OF COMPLEX EFFLUENTS

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ABSTRACT

The National Swedish Environment Protection Board has recently completed a research project: "Characterization of Industrial Effluents". Initially, analytical methods and laboratory tests, originally developed for single substances, were identified. Laboratories were then invited to participate, with their own methods, in ring tests with a variety of industrial effluent waters. The studies indicated that a number of chemical and biological methods can be used to characterize complex industrial waste waters, with regard to their content of toxic, persistent and bioaccumulative substances. A frame program was established for the construction of test batteries adapted to various administrative regulatory purposes.

To date, this strategy has been applied by the authorities to several industries, resulting in valuable experience of cost-effectiveness and the usefulness in the administrative decision-making process.

Besides the studies on single industries, two other approaches have been applied, namely for the assessment of the impact of several industries on a common receiving water body, and for the evaluation of a whole industrial branch, i.e. kraft pulp mill bleachery effluents. In the last case, the studies have resulted in a ranking of processes and treatment methods to protect the environment.

1. Introduction

According to section five of the Swedish Environment Protection Act, anyone responsible for a polluting activity shall take those safety measures, accept restrictions of his activity and otherwise observe those precautions which may reasonably be required to prevent or remedy the nuisance. To make it easier to construe this clause, reference is made to a couple of helpful rules which say that, for purposes of passing judgement on measures and precautions, there are two starting points: (1) What is technically/economically possible; (2) What is the nature of the area that is exposed to disturbance. The pre-enactment legislative materials go on to say that there is no need to delay an intervention until damage has occurred. In other words, protective measures may be demanded if there are grounds for assuming that the emissions will give rise to injuries.

The demands for more or less far-reaching reduction of emissions from polluting activities are generally put forward by the National Swedish Environment Protection Board and the County Government Board.

In Sweden, the National Franchise Board for Environment Protection is the government agency for issuing permits and licenses. In several cases, however, it has postponed its final ruling, contending that it has not been provided with an adequate decision-making basis. The background to this has been the lack of adequate test results and the risk that greatly adverse economic consequences might result from the wrong kind of pollution-abatement investment.

The Environment Protection Board has thus been faced with the problem of finding useful tests that can be performed by commercial laboratories. In the past, recommendations have generally been too crude to encourage commercial laboratories to develope and/or specialize on a particular set of tests that can be used routinely in a biological characterization of complex industrial effluents. In order to overcome some of these problems the Board started the project "Characterization of Industrial Effluents".

The use of biological tests to characterize complex waste water effluents has, however, also been practiced in some other Swedish projects and in administrative decision-making. Some of these experiences will be dealt with briefly in this presentation.

2. Characterization of Industrial Effluents

In this project tests with aquatic animals and originally developed for testing single substances have been used to biologically characterize complex waste water from nine factories. The participating laboratories were free to choose among their own tests, from which they had good experience and considered to be best suited for the purpose of the project. Several species of fish, crustaceans and one mussel species were used and effects on mortality, reproduction, development, growth, physiology and behaviour were studied. The methods were then compared with respect to sensitivity, reproducibility and the need for resources to perform the tests. Recommendations for the selection of tests and their use in a stepwise procedure were given for the biological characterization of waste water.

In cooperation with the Technical Department of the National Swedish Environment Protection Board and representatives from different sectors of industry, four industries were selected;

A. An organic-chemical industry with discharges into sea water of sodium formiate, chlorinated hydrocarbons etc after biological treatment. Produces vinyl chloride and polyvinyl chloride.

B. A textile plant discharging a number of chemicals and dyes into a small river after municipal biological and chemical treatment. Dilute rinsing water is discharged without purification.

C. A fine chemicals industry with a differential production of chemicals such as surfactants, organic chelating agents etc. Waste water is discharged to the municipal sewage system and after biological treatment into sea water.

D. An oil refinery with a production of 400-500 different qualities of oil, petrol and asphalt. The waste water is treated in a sequence of external purification stage including API separators and biological treatment.

For the purpose of intercalibration another 10 waste water were tested: kraft pulp mill (4), sulphite pulp mill (1), oil refinery (1), organic-chemical industry (2), chemical industry based on cellulose (1), mine (1).

As a result a list of suitable basic tests was established (Table 1) and a list of potentially useful methods in an expanded (confirmatory) investigation (Table 2).

Table 1

Suitable basic tests for characterization of industrial waste water (from Granmo et al. 1984).

Parameters		
Fish		
survival	Gasterosteus aculeatus (M,B,L)	96-hr
	Alburnus alburnus (B,L)	"
	Salmo gairdnerii (B,L)	"
	Brachydanio rerio (L)	"
reproduction	Brachydanio rerio (L)	
	Clupea harengus (M,B)	
	Phoxinus phoxinus (B,L)	
Crustaceans		
survival	Crangon crangon (M,B)	96-hr
	Daphnia magna (L)	48-hr
	Nitocra spinipes (B)	96-hr
reproduction	Nitocra spinipes (B)	
	Daphnia magna (L)	
Molluscs		
byssus test	Mytilus edulis (M,B)	

M = marine water, B = brackish water, L = limnic water

Table 2

Examples of test methods that might be used in an expanded investigation. (from Granmo et al. 1984).

Parameter		
Fish	growth	Phoxinus phoxinus (B,L)
	physiology	Perca fluviatilis (B,L)
		Salmo gairdnerii (B,L)
		Platichtys flesus (M,B)
	"rotatory flow"	Gadus morrhua (M)
		Perca fluviatilis (B,L)
	reproduction	Gadus morrhua (M)
	"late effects"	Brachydanio rerio (L)
Molluscs	reproduction	Mytilus edulis (M)
	growth	Mytilus edulis (M)
Echinodermata	reproduction	Echinus esculentus (M) *

M = marine water, B = brackish water, L = limnic water

* not tested in this project

3. Related projects

3.1. Cellulose Industry

During the first half of the 1970s the effort to protect water resources from pulp industry effluents was concentrated on the reduction of biochemically degradable material (BOD) and suspended material. This was relevant as long as oxygendeficiency or effects of fibrous matter on sea beds could be suspected. The work on reducing these effects has also been progressive, but today we have reached the point where further decreases in these parameters are becoming very expensive and yield only minor improvements in the environment.

In recent years, however, there has been a growing concern about the release of halogenated organic compounds from bleaching processes to the environment. For Sweden as a whole the emissions are calculated to be about 200 000 tons of chlorinated organic material per year. In terms of total organically bound chlorine (TOCl), this corresponds to about 15.000 tons per year and represents more than a hundred compounds. TOCl is an example of chemical parameters in the characterization program for pulp industry. Substantially more has become known about the chemical composition and biological effects of emissions from a project sponsored by the Swedish Pulp and Paper Association and the Swedish government. The project is named "Environmentally harmonized production of bleached pulp" and besides the attempts to chemically characterize the various compounds in the emissions, a variety of toxicological studies have also been performed. The latter studies include acute and chronic tests on fish, invertebrates and algae, studies on microcosms and Ames' test. Some of the results are summarized in Table 3, which contains a ranking of alternative processes and treatments in the production of bleached softwood kraft pulp.

The project "Environmentally harmonized production of bleached pulp" is largely completed, but several subprojects are continuing. Furthermore, the National Swedish Environment Protection Board has started a new five-year project: "Environment/-Cellulose", which seeks to clarify effects of bleached effluents on the aquatic environment. The project was started in

1982 and is thus not yet at the reporting stage. The research strategy was very much inspired by a former project in which results from laboratory tests were compared with results obtained from fish exposed to more or less complex metal-containing effluents in the field. As an outcome of this project, the use of a selected battery of clinical methods to estimate the pollution load and the status of exposed fish was encouraged.

The chemical characterization of bleached pulp effluents is an extremely difficult task to solve, and even with today's best analytical techniques only about 10 per cent of the effluent compounds have been determined. In such a case the role of the biological tests for classifying the effluents becomes extremely important for the choice of alternative processes and treatments to protect the environment. The project therefore aims at finding a set of biological tests that are particularly useful for the characterization of pulp effluents.

3.2. Environmental Impact Assessment

Most of the Swedish petrochemical industries are localized in Stenungsund, situated on the Swedish west coast 40 km north of Gothenburg. In 1982 the Swedish government decreed that there should be an extensive investigation of possible environmental effects in the region.

For the past 20 years, the control program has concentrated on estimations of fish stocks, bottom fauna, algae and sediment parameters. This program was, however, considered unsatisfactory for the assessment of any environmental impact in the receiving water body. It was thus suggested that a complemen-

tary battery of biological tests should be applied and that the results should be combined with the results from a chemical characterization (i.e. content of toxic, persistent and accumulating components in the wastes).
The program can be briefly summarized as follows:

1. Biological field studies (continuation of existing program)

2. Chemical characterization of wastes (content of toxic, persistent and accumulating substances), Fugacity calculations

3. Biological characterization of wastes.

Fish tests	96-hr LC50 (several species)
Crustacea tests	96-hr LC50 (Nitocra spinipes)
Algae tests	
Ames' test	
Bacteria, bioaccumulation and degradation tests (according to OECD)	
Tests with higher plants (Allium sp.)	

The data available at present indicate that the new strategy is helpful in the ranking of the most polluting industries, and consequently where to spend the efforts to improve the environment in the region.

Applications in administrative decision-making

A number of cases in which the biological and chemical characterization of the effluents has been important have already been heard by the Franchise Board.

In the following, two examples are given to illustrate how the data can be used by the administration.

1) Recently a polyethylene plant (UNIFOS) i Sweden considered an expansion for which, according to Swedish legislation, it needed a legal permit. The Environment Protection Board then asked for a biological characterization before making a decision. The caracterization showed that the acute and long term toxicity was low, the degradability was about 60 % but one component of the waste water showed a tendency to accmulate. Other information of importance was that the water quantities were small, the main discharge was to the atmosphere and the waste water passed a biological treatment plant before reaching the receiving water. With this information as a background the Franchise Board made the following decision: the amount of water discharge can be doubled but the industry must find and eliminate the components that have a tendency to accumulate and must report back the results within three years.

2) A manufacturer of organic chemicals (BEROL) wanted to tear up a former injunction to build a system for biological degradation of their wastes. The Environment Protection Board then advised some biological tests for characterization of the effluents. The results made the industry to suggest some process modifications to overcome the pollution problems. The Franchise Board then decided that these process modifications were acceptable as a substitute for the biological degradation system but that all measures had to be taken in agreement with the Technical Department of the Environment Protection Board. The Franchise Board has thus in these cases paid considerable attention to bioaccumulation and persistance in making its

decision. This marks a shift in policy from simply considering toxicity, BOD etc.

Experience of chemical and biological characterization of industrial effluents in Sweden must be evaluated in relation to the existing Swedish legislation and the system for permit-issuing and licensing. The test batteries are supposed to be used with flexibility and relate to administrative needs, economy, and the kind of effluents and the character of the receiving water body. Therefore a test program is always designed in cooperation between the administrative bodies, environmental scientists and, as far as possible, with the industry involved. However, the present situation is far from optimal and several problems have been located, such as: 1) Difficulty in finding performing laboratories 2) Communication problems between administration and laboratory staff 3) Insufficient competence within the decision-making bodies to interpret the test results. 4) Occasional difficulty in convincing the industry that a particular characterization program is useful and necessary.

With all this in mind, I do not intend to suggest that any other country should apply our system, which is peculiar to Swedish conditions. Nor is it yet an ideal product. The use of chemical and biological characterization is, on the other hand, to be encouraged to support better decision-making and to protect the enviroment from industrial effluents.

LITERATURE

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TABLE 3 RANKING OF ALTERNATIVE PROCESSES AND TREATMENTS IN THE PRODUCTION OF BLEACHED SOFTWOOD KRAFT PULP EVALUATED ON THE BASIS OF RESULTS OBTAINED BY MEANS OF CHEMICAL AND BIOLOGICAL TEST METHODS (FROM ANONYMOUS, 1982).

RELATIVE ENVIRON-MENTAL IMPACT.	ALTERNATIVE	COMMENT
HIGH	C_{90}/D_{10} EHDED	PRESENT TECHNIQUE
	C_{90}/D_{10} EHDED	IMPROVED WASHING COMPARED TO PRESENT TECHNIQUE
	C_{90}/D_{10} EHDED + UF (E_1)	ULTRAFILTRATION OF E_1 EFFLUENT
	$O(C_{85} + D_{15})$ EDED	OXYGEN BLEACHING TO KAPPA NUMBER 20, WASHING TO 10-12 KG/T_{90} AS COD
	C_{90}/D_{10} EHDED + UF (E_1) + OZ	OZONE TREATMENT OF PERMEATE AND C EFFLUENT
	$O(C_{85} + D_{15})$ EDED	OXYGEN BLEACHING TO KAPPA NUMBER 20, WASHING TO 4-6 KG/T_{99} AS COD
	C_{90}/D_{10} EHDED + AERATED LAGOON	TREATMENT OF TOTAL EFFLUENT IN AERATED LAGOON
	C_{90}/D_{10} EHDED + ION EXCHANGE	ION EXCHANGE OF TOTAL EFFLUENT
	D_{85}/C_{15} EDED	
	$O(C_{85}/D_{15})$ EDED + AERATED LAGOON	OXYGEN BLEACHING TO KAPPA NUMBER 20
LOW	$O(D_{85}/C_{15})$ EDED	OXYGEN BLEACHING TO KAPPA NUMBER 17

C_{90}/D_{10} = 90 % CL_2, 10 % CLO_2; E = EXTRACTION WITH ALKALI; H = HYPOCHLORITE SEQUENCE; O = OXYGEN SEQUENCE; OZ = OZONATION

PRINCIPLES FOR COMPOSING BIOLOGICAL-CHEMICAL TEST SYSTEMS FOR COMPLEX EFFLUENTS.

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ABSTRACT

Hitherto, the concern for possible environmental effects due to aqueous industrial discharges has been directed towards clearly observable effects such as eutrophication and fish kills. However, the development of environmental toxicology and chemistry has made it clear that also more subtle and potential long-term effects have to be taken into account in the regulatory work.

The Swedish project "Characterization of industrial effluents" has therefore evaluated a number of chemical and biological tests which, in combination, can be used for the proper assessment of the environmental impact of aqueous effluents. Thus, the test programme should be based on both the chemical characterization of the water and studies on toxicity and persistence.

The chemical characterization of waste water samples consists of determination of group parameters responding to chemically related compounds and quantification of known or suspected biologically active compounds. Toxicity studies include the estimation of both acute toxic effects and chronic effects (eg reproduction disturbances and genotoxicity). The persistence is characterized by microbiological degradation and by bioaccumulation potential.

Besides the results of the individual tests the overall considerations are also based on information of the particular industrial processes and the character of the receiving waters. It is emphasized that the investigation should be performed in a step-wise manner. That is, the results of the initial - and relatively general and simple - tests should determine the choice and design of more sophisticated (and expensive) tests, if needed. Test results, evaluated and compiled by persons well experienced in the field of ecotoxicology can then serve as a base for decisions to decrease or remove negative biological effects.

INTRODUCTION

The work which has been devoted to reduce negative effects of industrial aqueous discharges has to a large extent been directed towards the reduction or elimination of clearly documented effects. Examples of such effects are eutrophication caused by the release of nutrients and extensive fish kills due to toxic discharges. Care must, however, be taken to detect and reduce also long term effects, which are often not easily observed and sometimes even masked by the acute toxic effects. Special consideration should be given to persistent and bioaccumulating substances which may extend possible effects also to remote parts of the receiving waters.

The need for a scientifically based ecotoxicological approach for administrative decisions concerning the regulation of industrial discharges resulted in a research project entitled "Characterization of industrial waste waters" (1). The aim of the project was to develop and evaluate ecologically relevant methods, which could be applied for administrative purposes. Examples of such purposes are how to assess the ecotoxicological impact of waste water effluents when establishing new factories or modifying processes at existing plants. Other applications involve evaluations of different types of waste water treatment processes for the reduction of toxic compounds and chemical and/or biological monitoring of waste water streams.

It was realized that a single test - even a very sophisticated one - could not fully reflect the environmental impact of a complex waste water sample. Within a given financial frame, it was decided that a battery of relatively simple tests, reflecting different properties of the waste water samples would be a better choice. The principles for composing such a biological--chemical test system for complex aqueous effluents will be given here. The application of these principles on different types of industrial waste waters will be discussed in the paper presented by Dr Bengt-Erik Bengtsson.

The fundamental principle when designing such a test system is that a waste water sample can be characterized for its potential environmental impact by chemical analysis (including both group parameters and determination of specific compounds), toxicity (acute and sublethal effects) and persistence (resistence against microbiological degradation and bicaccumulation).

A complete set-up of tests, covering all these aspects tend to be rather expensive and time-consuming. Both from financial and scientific points of view it is recommended that the tests are carried out in a step-wise manner, ie the results of the initial - and the relatively general and simple - test should determine the choice and design of more sophisticated (and expensive) tests, if needed.

We have found that a combination of chemical characterization and biological tests is very valuable. Our experience in Sweden is that information often exists which may at least partly describe the content of an industrial waste water sample from a chemical point of view, while the biological activity is usually less well known. Chemical information can often be obtained in terms of product use pattern, additives used and chemical analyses previously carried out. Such information is also valuable when evaluating biological effects if the toxicity of the individual compounds is known. However, such information is usually insufficient when attempting to predict interactive effects.

CHEMICAL CHARACTERIZATION

The relatively simple chemical characterization of a waste water sample consists of three types of investigations (Figure 1). Firstly, the organic content of the waste water sample should be known. An approximate value can be determined using COD (Chemical Oxygen Demand) or TOC (Total Organic Carbon) analyses, preferably after fractionation of the waste water sample using ultrafiltration. In such a way, values of both the low molecular ($MW < 1000$) and the high molecular ($MW > 1000$) content can be approximately estimated.

Secondly, methods using group parameters, eg total amount of oil or extractable organohalogens, are often valuable and should be chosen in relation to the specific industrial activity.

If the waste water is known to contain specific compounds (eg heavy metals) - or classes of compounds (eg chlorophenolics)-

representative for the production, a selected number of such components should be monitored to measure the concentration of known toxic pollutants but also to ensure that the sample, which is also used for the biological characterization, is representative for the industrial activity.

More comprehensive investigations, if necessary, usually involve characterization by combined gas chromatography/mass spectrometry and high performance liquid chromatography for polar and high boiling compounds.

SCREENING TESTS

- DETERMINATION OF THE APPROXIMATE TOTAL AMOUNT OF RELEASED SUBSTANCES (EG COD)
- DETERMINATION OF PARAMETERS, RESPONDING TO CHEMICALLY RELATED COMPOUNDS (EG TOTAL AMOUNT OF OIL, ANJON ACTIVE SUBSTANCES)
- DETERMINATION OF CHEMICALLY DEFINED SUBSTANCES, TYPICAL FOR THE SPECIFIC INDUSTRIAL ACTIVITY (EG HEAVY METALS/MINING, CHLOROPHENOLS/PULP MILLS)

COMPREHENSIVE TESTS (EXAMPLES)

- CAPILLARY GAS CHROMATOGRAPHY/MASS SPECTROMETRY
- HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING SPECIFIC DETECTION (EG FLUORESCENS OR ELECTRO CHEMICAL DETECTION)

Figure 1. Chemical characterization.

BIOLOGICAL EFFECTS

The strategy for the biological characterization has been to apply a few, relatively simple tests, each reflecting different types of toxicity in order to detect effects on different organisms at different trophic levels (Figure 2). Thus, the biological activity of the waste water sample is characterized both by its acute and chronic toxicity, respectively. Fish, usually a local inhabitant, ie bleak (Alburnus alburnus) or the sebra fish, together with the crustacean species Daphnia magna and Nitocra spinipes (2) have extensively been employed to monitor acute toxicity.

Studies on sublethal effects have been concentrated on reproduction disturbances and inhibition of the growth. The reproduction disturbances can conveniently be studied using the previously mentioned two crustaceans. Primary production has been measured using algae and/or higher plants.

The screening for genotoxicity, which is of particular importance for receiving waters used for drinking water production has been carried out using Ames' mutagenicity test, with or without metabolic activation (eg mammalian liver microsomes).

In some cases, more thorough investigations may be necessary. Such investigations may consist of comprehensive studies on fish (eg growth, physiology), molluscs (eg growth, reproduction) and

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SCREENING TESTS

- ACUTE TOXICITY
  FISH, CRUSTACEAN EG SEBRA FISH
  DAPHNIA MAGNA
  NITOCRA SPINIPES
- REPRODUCTION DISTURBANCES
  FISH, CRUSTACEAN EG SEBRA FISH
  DAPHNIA MAGNA
  NITOCRA SPINIPES
- PRIMARY PRODUCTION
  ALGAE, HIGHER PLANTS EG CHLORELLA
  ALLIUM CEPA
- GENOTOXICITY
  AMES BACTERIA TEST
  WITH AND WITHOUT MAMMALIAN MICROSOMAL ACTIVATION

COMPREHENSIVE TESTS

- DIVERSITY
- REPRODUCTION, GROWTH
- PHYSIOLOGY
- BEHAVIOUR

Figure 2. Biological effects.

algae (diversity). It should be stressed that, if possible, the organism used for the biological tests previously discussed should be as representative as possible for the particular waters into which the waste water is discharged.

A fast and non-expensive test, which recently has grown in popularity is the so called Microtox test. The test is based on the measurement of the quenching of bioluminiscence light produced by certain marine micro organisms. The evaluation of the microtox test on waste water samples is still underway but it should be useful in any case to monitor the toxicity of waste water effluents to ensure the representativity of waste water samples used for more sophisticated and expensive biological testing.

PERSISTENCE

When assessing the environmental impact of complex mixtures, the toxicity should be regarded in view of the persistence of the toxic components. If a substance is not easily degradable, the toxicity will in such a case be extended to the remote parts of the receiving waters. The estimation of persistence includes both the application of relatively conventional tests, eg respirometric tests (monitoring BOD during a test period of four weeks) and die-away tests. The screening for potential bioaccumulating compounds involves the use of reversed phase thin layer chromatography for the isolation and characterization of compounds with high log P_{ow} values (3).

More intense investigations will usually include biodegradation tests with a simultaneous monitoring of individual compounds by chemical analyses. For more thorough studies on bioaccumulation, the determination of individual compounds in fish or bivalves exposed to waste waters in laboratory continuous-flow--system is recommended.

MICROBIOLOGICAL DEGRADATION

SCREENING TESTS

- RESPIROMETRIC TESTS (28 DAYS)
- DIE AWAY TESTS

COMPREHENSIVE TEST (EXAMPLE)

- MONITORING SPECIFIC COMPOUNDS (CHEMICAL ANALYSIS) DURING DEGRADATION TESTS

BIOACCUMULATION POTENTIAL

SCREENING TEST

- SCREENING FOR SUBSTANCES WITH HIGH LOG P_{OW} WITH REVERSED PHASE THIN LAYER CHROMATOGRAPHY

COMPREHENSIVE TEST (EXAMPLE)

- MONITORING THE UPTAKE OF SPECIFIC COMPOUNDS IN AQUATIC ORGANISMS

Figure 3. Persistence.

GENERAL CONSIDERATIONS

All tests previously discussed should be carried out by experienced laboratories which have adopted some kind of laboratory quality criteria, eg GLP (Good Laboratory Practice eg according to OECD's Chemicals Testing Programme). The laboratories should be encouraged to take part in intercalibration exercises and/or harmonizing activities.

The choice of the individual tests when designing a test battery for initial hazard screening should be carefully considered. Some important criteria are given in Figure 4.

The need for a close co-operation between chemists and biologists has previously been stressed. Benefits of such an integrated collaboration are of both a principal nature - eg to relate biological activity to the presence of specific compounds - and of a practical nature - eg how to create test conditions which also include tests of hydrophobic substances (especially bioaccumulation tests).

- RELEVANCE
- COST EFFICIENCY
- SENSITIVITY
- SELECTIVITY
- REPRODUCIBILITY
- RUGGEDNESS
- EASY TO USE
- EASY TO STANDARDIZE OR HARMONIZE WITH SIMILAR METHODS

Figure 4. Pertinent features for a method used for biological/-/chemical characterization of waste waters.

The results obtained in the different tests for a specific waste water sample can be stored as hard copies as well as in a computerized information system, eg the CETIS data base (4). In such a way data retrieval and information analysis are facilitated for both the authorities and scientists. In Sweden activities in this area are underway.

As pointed out in Figure 5, the data obtained experimentally in the different tests are not the only data pertinent for an integrated assessment of the environmental impact of waste water effluents. Examples of other factors to be taken into consideration are the character of the receiving waters and information concerning the industrial processes, including products and by-products, raw materials and additives used. Information can also be obtained by literature searches and documented knowledge about similar industrial processes. In some cases such documentation may be sufficient for serving as a base for appropriate decisions and thus no experimental tests are needed.

However, there are also occasions where more comprehensive or specific tests are needed. Examples of such tests are when industrial activities are to be located in regions of special importance (used for eg commercial fishing, recreation, drinking water production etc) or at occasions when representatives for the authorities and the industry, respectively, disagree in an important matter. An example of a relatively comprehensive test battery is shown in Figure 6.

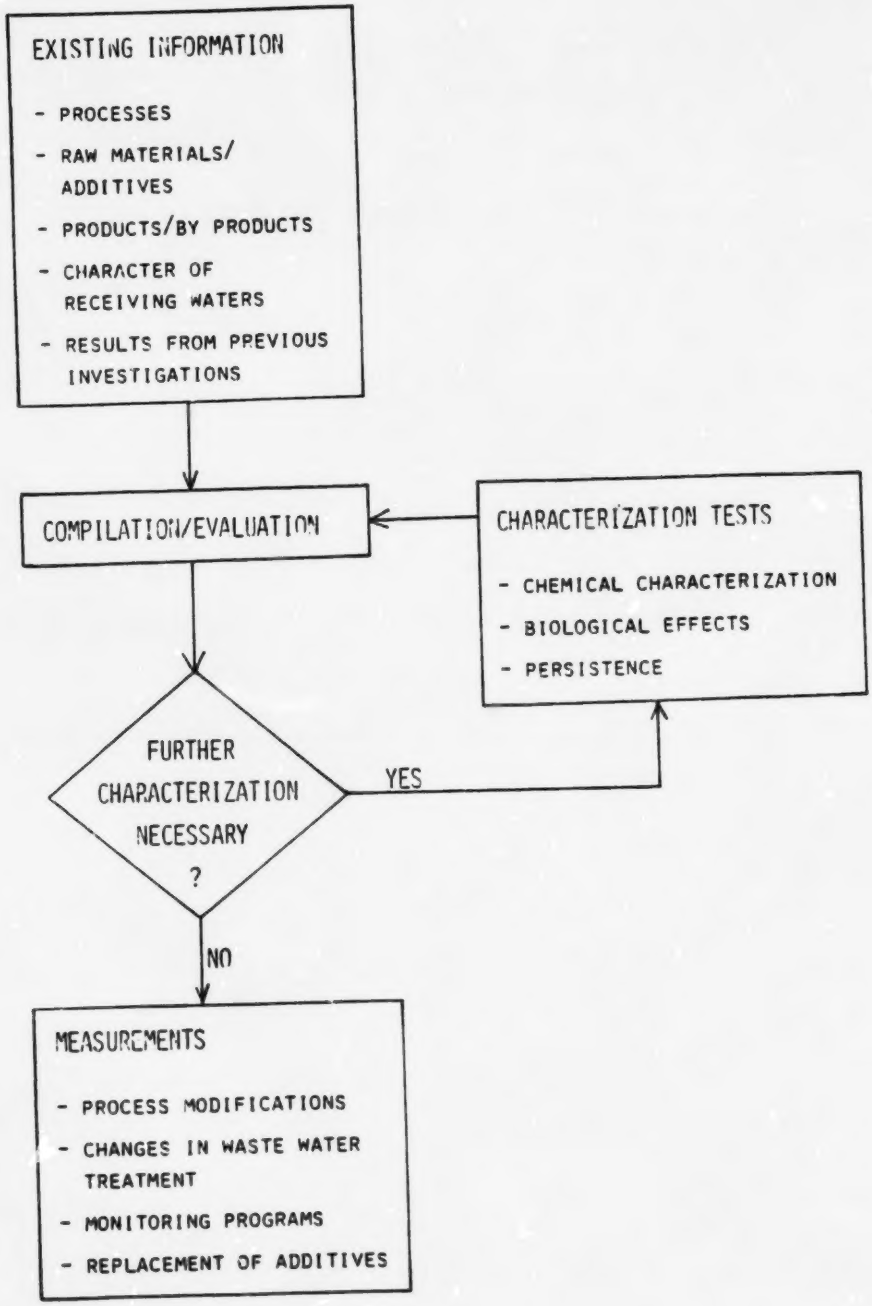
EXISTING INFORMATION
- PROCESSES
- RAW MATERIALS/ ADDITIVES
- PRODUCTS/BY PRODUCTS
- CHARACTER OF RECEIVING WATERS
- RESULTS FROM PREVIOUS INVESTIGATIONS
COMPILATION/EVALUATION
CHARACTERIZATION TESTS
- CHEMICAL CHARACTERIZATION
- BIOLOGICAL EFFECTS
- PERSISTENCE
FURTHER CHARACTERIZATION NECESSARY ?
YES
NO
MEASUREMENTS
- PROCESS MODIFICATIONS
- CHANGES IN WASTE WATER TREATMENT
- MONITORING PROGRAMS
- REPLACEMENT OF ADDITIVES

Figure 5. Components needed for the characterization of waste water effluents.

1. CHEMICAL CHARACTERIZATION

GROUP PARAMETERS
- TOTAL AMOUNT OF OIL (INCLUDING APPROXIMATE ESTIMATION OF AROMATIC CONTENT)

DETERMINATION OF SPECIFIC COMPOUNDS
- NICKEL, VANADIN (AA)
- POLYAROMATIC COMPOUNDS (GC OR HPLC)

2. BIOLOGICAL EFFECTS

- ACUTE TOXICITY / FISH
- ACUTE TOXICITY / CRUSTACEAN
- PRIMARY PRODUCTION / ALGAE
- GROWTH / MUSSEL

3. PERSISTENCE

RESISTANCE AGAINST MICROBIOLOGICAL DEGRADATION
- RESPIROMETRIC TEST WITH THE SIMULTANEOUSLY MONITORING OF POLYAROMATIC COMPOUNDS (CHEMICAL ANALYSIS)

BIOACCUMULATION
- BIOCONCENTRATION OF POLYAROMATIC COMPOUNDS IN MUSSEL

Figure 6. Characterization of refinery waste waters.

It may be difficult or even impossible to extrapolate test results or the final assessment for the prediction of the biological impact in the particular receiving water body.However, our experiences have shown that an integrated test battery is particular useful for carrying out relative assessments, ie the possibility to compare and rank the environmental impact of several waste waters. In such a way, an optimization can be made between cost and efficiency for specific measurements.

The final step in the assessment process is to compile and evaluate all data - both those experimentally obtained and other types of information. It should be stressed that this process is a most important step and should be carried out by people (usually consultants) well experienced in the field of ecotoxicology. During the planning, execution and compilation of the results, a close co-operation between the authorities, the consultants and the laboratories involved and representative for the individual industry has shown to be highly advantageous.

The compiled results, together with the final assessment, will then serve as a basis for administrative decisions. Adequate measurements can then be taken to reduce or remove negative effects with respect to what kind of measurements that are technically/financially feasible in the particular case.

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UTILITY OF EFFLUENT TOXICITY TESTS FROM THE U. S. REGULATORY PERSPECTIVE

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ABSTRACT

The U.S. approach to regulating wastewater discharges to surface waters combines two separate approaches: minimum technology requirements and more stringent requirements, where needed, based primarily upon demonstrating that additional controls are required to attain ambient quality criteria. Dischargers may challenge specific treatment requirements on the grounds that they are more stringent than necessary to meet ambient criteria. Therefore, the U.S. approach depends heavily on understanding cause-effect relationships and determining the highest discharge levels that will still provide water quality protection. For the last several decades we have pursued the development of numerical ambient quality criteria for individual chemicals and pollution parameters with the belief that such chemical criteria provide the best way to approach the cause-effect relationship and calculate discharge requirements. In recent years we have concluded that, for toxic effects, the chemical specific approach may not be the best means to assess effects because the effects of toxicants vary depending on the water matrix and interactions with other toxicants. Another problem concerns the difficulty in measuring uncommon chemicals and the lack of toxicological data for such chemicals. Because of these problems, the U.S. has begun to use toxicity tests to measure whole effluent effects and project ambient effects as a supplement to chemical specific regulation. This paper reviews the U.S. experience with regulation of toxicants, describes the current use of toxicity tests in the U.S., and presents what we consider to be the major issues associated with the use of toxicity tests to regulate the discharge of toxicants.

THE U.S. APPROACH

Companies and municipal sewage authorities which discharge wastewaters in the United States are obliged to comply with two independent requirements for pollution control. The first requirement is to install and operate equipment to meet municipal or industry-specific technology-based performance standards. These are uniform treatment requirements based on technological achievability and the economic impact to the industry segment resulting from compliance (e.g., the number of plant closures). Technology-based requirements are not based on water quality effects. The second requirement dischargers must comply with is based on protecting water quality. These requirements are, therefore, site-specific and depend on the level of quality desired for the water body and the amount of pollutants discharged in relation to the assimilative capacity of water body.

Prior to 1972, water quality standards issued by the State governments provided the only means to control water pollution. The technology-based performance standard approach was adopted nationally in 1972 because of the perceived failure of the water quality-based approach in achieving adequate pollution control. The failure was largely due to the difficulties and cost involved in showing that a water is degraded, difficulties in assigning responsibility to individual sources, and lengthy debates over control measures. However, the failures may have been due as much to the absence of effective enforcement mechanisms as the scientific difficulties associated with the water quality-based approach. Delay was an effective strategy for opposing treatment requirements and the water quality-based approach provided numerous opportunities to raise issues in order to delay action.

In 1972, the U.S. Congress enacted a nationwide permit system for setting and enforcing discharge controls. Since that time, many water quality-based requirements have been incorporated into discharge permits even though the emphasis has been on achieving compliance with the technology-based performance standards. For the most part, water quality-based requirements have been for the control of BOD, nutrients, and metals. We are now nearing completion of the technologybased aspect of our control system with effluent limitations guidelines having been written to control conventional and toxic pollutants in most categories of industry. Now we are preparing to shift to an emphasis on water quality-based controls with a special emphasis on toxic pollutants as the basis for more stringent limits in the areas that require better treatment than our nationwide technology standards.

Under our statutes water quality standards provide the basis for the water quality approach. The standards are issued by the 50 State governments and the Federal government has authority to veto and reissue standards that do not comply with the broad requirements of the Federal statute. Water quality standards consist of two provisions -- a "designated use" that defines the water quality management objectives for a water body, and specific criteria to achieve the designated use. Our original intention was to incorporate specific concentration criteria for every significant pollutant into water quality standards in order to remove much of the uncertainty formerly associated with the water quality-based approach. Once criteria are established, the procedure to determine treatment needs is to calculate a wasteload allocation based on attainment of the criterion during some worst case exposure condition. States may provide for a limited zone of mixing.

The successful application of water quality-based treatment requirements in the U.S. depends on being able to demonstrate that additional treatment is necessary to meet water quality standards and that requirements are not overly stringent. Dischargers may and often do challenge water quality-based requirements on the technical aspects of the analysis used to derive requirements. Therefore, under our legal structure, understanding the cause/effect relationships between pollution and water quality degradation is especially important because the analysis to determine discharge requirements may end up in a court of law.

EXPERIENCE WITH TOXICANTS

With the establishment of criteria for all significant pollutants as our goal, in the early 1970's the Federal Environmental Protection Agency (EPA) began to collect data and develop recommended water quality criteria. By the mid-seventies it became apparent that the existing data on toxic pollutants were inadequate and EPA began an intensive testing program. This effort culminated in 1980 with the publication of recommended criteria for the protection of aquatic life for 22 specific compounds and criteria for the protection of human health for 65 classes of compounds. At the same time we developed a method for deriving criteria that included minimum data requirements and procedures to modify the recommended criteria to account for site-specific factors.

From this criteria development effort we learned several things. First, the development of sufficient toxicological data to satisfy our own requirements for the minimum data set is very expensive. Development of a single criterion value costs $50 - 100,000 US. Secondly, all indications at the time led us to conclude that site-specific factors such as water chemistry have a strong effect on toxicity and that an opportunity should be provided to conduct on-site testing to modify the criteria. More recent data have shown that the concern about local factors may have been overstated, but this is still an issue. Finally, the State governments did not adopt our criteria as we had hoped. Many States have been reluctant to incorporate the EPA criteria into their standards because of concerns about the scientific validity of the criteria and the cost of monitoring. This concern was exacerbated by our own recommendations that the values be modified for site-specific factors, which could be an added expense for the States. Finally, the emphasis on completing the national technology requirements tended to place less priority on the water quality standards until this year.

LIMITATIONS OF THE POLLUTANT-SPECIFIC APPROACH

At the same time that we were developing criteria recommendations for toxicants a number of limitations of the individual toxicant approach became apparent. First and foremost is the importance of the mixture in changing the effects of toxicants. There are two factors responsible for complicating predicted effects from toxicants when they exist in complex mixtures. One factor is the physiological effect of multiple toxicants on organisms. Often the effects of different toxicants are additive but sometimes they are not and developing predictive models for this phenomenon does not appear to be a fruitful endeavor. The other factor that complicates predictions based on chemical criteria is matrix effects caused by effluent and receiving water constituents. We have seen many examples of toxic effects being altered significantly upon mixing with receiving waters and other effluents.

In addition to limitations resulting from complex mixtures there are problems stemming from the current ability to detect and measure a large number of potentially toxic pollutants. Even for those substances that we know are discharged, there are often not sufficient data on toxicological effects to base regulatory decisions. In an effort to facilitate data management, EPA has developed several extensive data systems. But there are still many potentially hazardous pollutants that have never been tested.

Because of the limitations inherent in depending exclusively on a pollutant-specific chemical approach, we began looking at biological techniques. Biological techniques include a broad spectrum of methods such as ecological field surveys and laboratory tests of effects. Ecological survey techniques are fairly well established and are occasionally used in a regulatory context. However, in the U.S. there is considerable reluctance to use field surveys because results are often ambiguous and subject to argument. The major regulatory experience with ecological survey techniques were waiver provisions for thermal pollution control requirements and ocean discharges of municipal waste. Millions of dollars have been spent on studies which were generally inconclusive. A second problem with ecological surveys is that it is difficult to establish treatment requirements to mitigate impacts detected -- especially in multiple source situations. For these reasons we have focused our attention on toxicity testing as a technique for regulation.

USE OF TOXICITY TESTS

Using toxicity tests to measure the effects of effluent dischargers overcomes several of the limitations of chemical testing. First, toxic effects are measured directly in a manner that incorporates both multiple pollutant effects and water chemistry or matrix effects. Secondly, an assessment of water quality impact using toxicity tests may be less costly than relying exclusively on chemical testing. And finally, a significant advantage is that dischargers often find toxicity test results more credible than an assessment based on chemical projections. Attaining the cooperation of the discharger is more likely if a toxicity problem is demonstrated.

We are moving very aggressively to incorporate the use of effluent toxicity as a limitation parameter in discharge permits. Because tests are conducted on effluent samples and the effects of dilution water can be factored in the analysis, we believe effluent toxicity requirements are as enforceable as any other permit limitation. Our greatest concern for enforceability is associated with method standardization and quality control. However, the data we have on reproduceability show better results for effluent samples than pure compounds and acceptable overall precision.

Because of the large number of sources in the U.S., changing information, and the required periodic renewal of permits, we see the regulation of surface water discharges as a periodic process consisting of three steps: screening, assessment, and control. In many of our States an additional step of setting new water quality standards for toxic chemicals and reevaluating the attainability of some established water quality standards is worked into the process. EPA is developing specific procedural recommendations for implementing this process. The main features of our recommended procedures are described below.

In the screening step, the objectives are to gain understanding while minimizing costs and minimizing false negative results. A quick assessment of effluent exposure is the most important element coupled with whatever existing data are available for the discharge. One thing we have learned using toxicity tests is that effluent toxicity is not predictable by looking at commonly considered factors such as industry type or effluent chemistry. Therefore, we are very cautious about "a priori" estimates of effluent toxicity. The screening criteria focus primarily on dilution.

The second step, impact assessment, has three purposes: to determine or confirm that water quality impact exists; to develop effluent variability data if a dynamic exposure model will be used to develop controls; and to develop data on species sensitivity to avoid using an uncertainty factor in the development of controls. It is important to note that we do not view data collection in order to accomplish these objectives as a prerequisite to control. Effluent limits can be developed without going through the assessment step but uncertainty factors may have to be used to account for incomplete information about the effluent and certain types of exposure modelling may not be possible.

When an assessment will be conducted, EPA believes that a tiered testing protocol should be used to reduce costs to dischargers. At the end of each testing tier the results are evaluated to determine if impact is projected. If impact is projected a choice is made to develop better data or to institute controls. However, in projecting impact, uncertainty factors are applied to account for what we consider to be incomplete data. If sufficient data exist, the uncertainty factors are not used in the projection of impact. Figure 1 describes the uncertainty factors and the data requirements to eliminate them from the assessment.

The final step is control. Water quality-based discharge requirements are derived from a wasteload allocation. A wasteload allocation is used to compute an allowable effluent load based on assimilative capacity and the water quality criteria. Loadings are divided among multiple dischargers in this process. There are several methods to calculate wasteload allocations and some permutations are as follows: (1) by assuming steady state conditions or by using dynamic modelling methods to assess duration and frequency of projected instream effects; (2) by accounting for fate and transport or by assuming pollutants are persistent; or (3) based on effluent dispersion or assuming completely mixed conditions. We have recently discovered that for toxic pollutants the duration of concentrations above effect levels and the frequency of occurrence are the critical factors in projecting impact and developing controls. This is because toxic effects are more time-dependent than effects such as eutrophication, oxygen depletion, or bioaccumulation.

After the wasteload allocation is completed the requirements are incorporated into the discharger's permit. We have only recently carefully examined the effect of current practices and have found a number of inconsistencies within commonly used processes for establishing permit limits from wasteload allocations. These inconsistencies arise from different assumptions for allowable exceedance rates and averaging of measurements.

A common complaint about using effluent toxicity to establish discharge requirements is that the dischargers do not know how to treat their wastewaters to comply with such requirements. We recognize that the development of control strategies is more difficult for toxicity requirements than for chemical requirements but we believe that it is no less feasible. Our approach is to provide time for a discharger to conduct a study we are calling a toxicity reduction evaluation (TRE). Conducting a TRE, as with any control activity, is the responsibility of the discharger. We now have several examples of companies successfully completing a TRE and achieving compliance with toxicity permit requirements.

CURRENT USE OF TOXICITY TESTS

There are currently 65,000 facilities within the US water pollution control permit program. Of these, 7500 are classified as "major" facilities. We estimate that there are several hundred permits with some kind of toxicity testing requirement and that between 50 and 100 permits contain effluent toxicity discharge requirements. The use of effluent toxicity as a discharge characteristic within the regulatory program has been hampered for several years by confusion over the Federal policy. A policy was finally developed and issued this year and we expect that use of toxicity as a regulatory parameter will increase.

Many of our States are currently developing capabilities to conduct toxicity tests. It is the EPA's firm position that dischargers should bear the burden of effluent characterization, impact assessment, and compliance with their permit. However, the States need to have a capability to perform the tests for compliance inspection and enforcement purposes. Also, many States want to complete studies of their own to convince themselves that toxicity is a useful assessment and regulation parameter.

The U.S. Federal government is working to provide technical assistance to the States through research and guidance. We are preparing a technical support document that provides several specific recommendations for implementation of the approach. Our field laboratories are expanding their efforts in toxicity testing and, in those states where EPA runs the permit program, our permit writers are beginning to require testing and utilize toxicity as a permit limitation parameter.

MAJOR ISSUES

There are several issues that we see as critical to the cost effective use of toxicity testing as a regulatory tool. The first relates to the number of test species that should be used to characterize effluent toxicity. The presumption is that effluent toxicity, if appropriately measured, provides a projection of community response to an effluent discharge. Our recommendation for effluent toxicity evaluation is that any species may be used provided several different types of organisms are tested and the most sensitive used to project community response. This recommendation presumes that the natural presence of the organism in the receiving water is not important. This recommendation for effluent testing is quite different from the data requirements for single chemical criteria and the procedures for modifying these criteria, in that criteria may be modified based on species presence. Our preliminary analyses of species sensitivity tend to support this position for whole effluent evaluation. But a more important consideration is that cost constraints argue against collecting as complete a data base for individual permits as for the national recommended criteria.

The second major issue is assessing the variability of a discharge. Because toxic discharges often result from spills or intermittent process discharges, it can be expected that the toxicity of an effluent will vary significantly with time. This factor affects both the method of sampling that should be used and the frequency with which samples should be collected and tested. It seems reasonable to approach the problem by guessing the variability that might be expected from the facility and tailoring sampling accordingly. But for toxicity, we have little information to even begin to guess at potential variability.

The third issue is persistence. Many dischargers raise the fate of toxic discharges as an issue arguing that toxic pollutants are quickly degraded or otherwise removed from the system. We have recommended employing a simplifying assumption that toxic pollutants are persistent unless information is available to model toxicity degradation. This is based on the consideration that most water quality standards require that safe concentrations exist outside of a relatively small mixing zone. A well treated effluent should not degrade significantly within a small mixing zone. A second consideration supporting our recommendation is that any rapid changes that would occur are duplicated within the toxicity test. Nonetheless, we need methods to assess easily the persistence of toxic effects after discharge.

Costs are a major concern in any regulatory program. In the U.S., the costs for a contract laboratory to conduct toxicity tests have started to come down as a result of increasing demand. We are confident that prices will eventually become quite reasonable as testing becomes routine. There is a very urgent need, however, for standardized protocols and quality assurance procedures.

One area that we are not at all comfortable about is assessing the potential for human health impacts. Few chemicals have formal tolerance levels for intake. The available screening bioassays that could be used to test complex mixtures for health effects are costly and provide inconclusive results. We are recommending that effluents be subjected to HPLC separation and chemical analysis when potential hazard from bioaccumulative pollutants is suspected. But in many cases it will be impossible to identify compounds or estimate risk levels for compounds that are identified.

The last major issue related to regulatory use of toxicity is determining the treatability of wastewater dischargers to achieve toxicity requirements. As previously described, in the U.S. it is the discharger's responsibility to develop control strategies and we believe most companies can accomplish the task. However, it is the regulatory agency's responsibility to provide guidance and methods. EPA's research efforts in this area have been sporadic and we do not have much guidance to help companies grappling with toxicity control requirements. We are planning to document case histories next year and develop guidance for dischargers.

SUMMARY

The U.S. is moving toward increased use of effluent toxicity as an assessment and control parameter. However, several basic questions remain unanswered. Our requirements for a quantitative approach to water quality impact assessment and control result from our regulatory system in which legal defensibility depends upon an understanding of the cause/effect relationships for water quality degradation. The issues that have been identified as most critical for regulatory use should be resolvable through additional research and programmatic experience. The Organization for Economic Cooperation and Development, in deciding to explore these issues, is providing an opportunity for international research, discussion, and consensus building that will significantly aid the application of biological assessment techniques in the control of water pollution.

Factor	Purpose	Value	Requirement
Effluent Variability	To correct for insufficient data in assessment step only	10-100	Factor may be removed after completion of a testing program to assess variability
Species Sensitivity	To correct for insufficient data in assessment and control steps	10	Factor may be removed if 3-5 species tested and the most sensitive used
Acute to chronic ratio	To adjust between acute & chronic toxicity in assessment and control steps	10-20	Use depends on type of test used for monitoring and the effects of concern

Figure 1. Uncertainty and adjustment factors for effluent impact prediction

POOR

QUALITY

ORIGINAL